PLANT CELL BIOLOGY AND DEVELOPMENT

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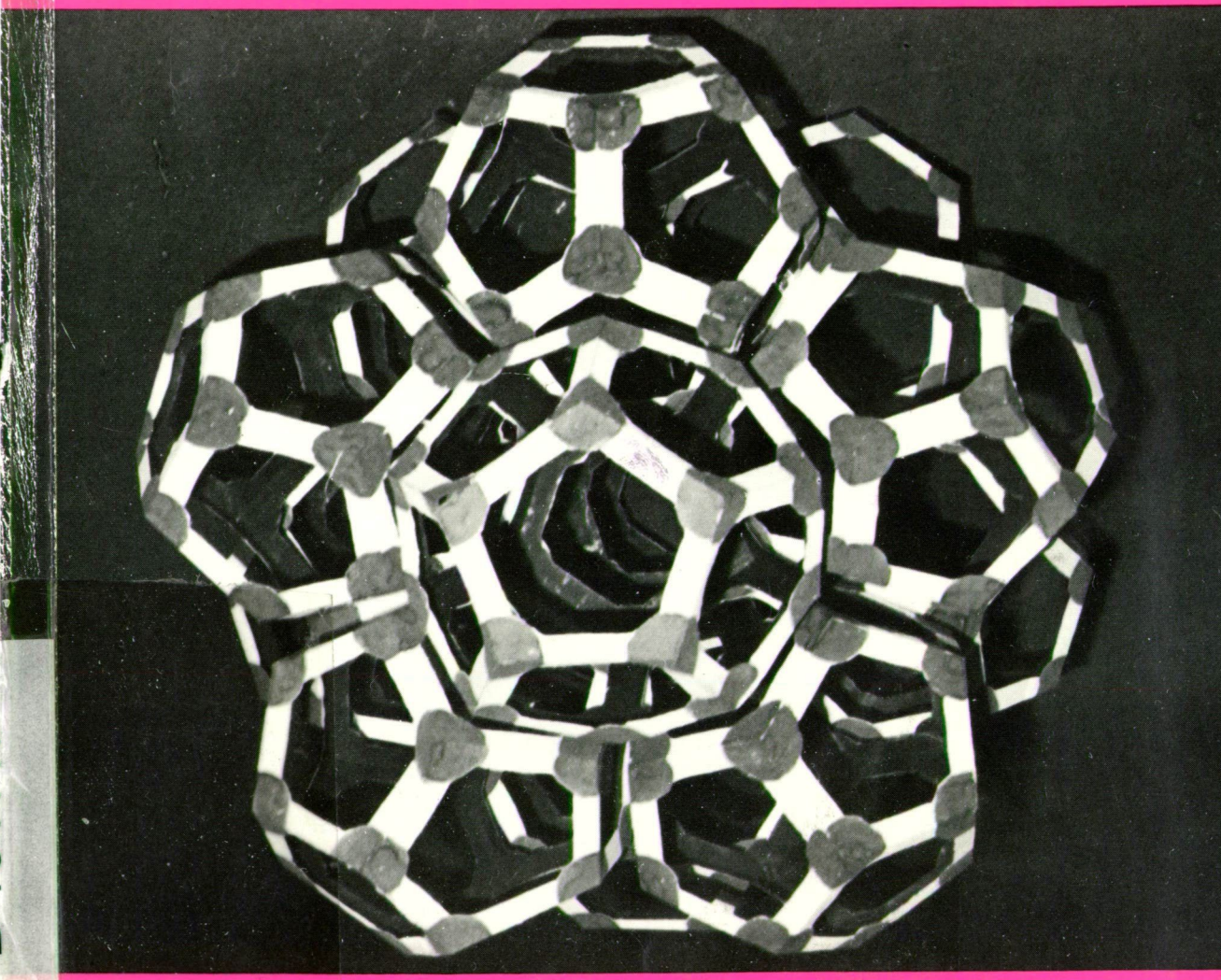

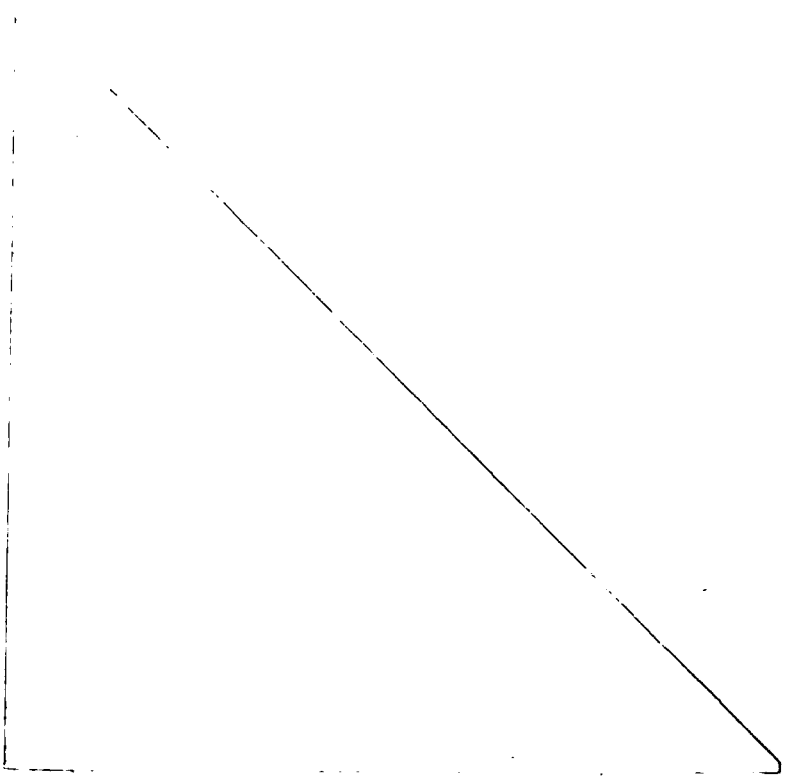

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3

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Contents

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Preface

It was nearly one year ago that the first number of the publication series of our Laboratory appeared. Followed quite quickly by the second one. The opinions concerning this effort were generally very favourable.

But to avoid some misunderstanding it is necessary to emphasize the following:

Plant Cell Biology and Development is not a scientific journal or review, but a periodical publication of the laboratory, but publishes exclusively papers from famous scientists of the world who have been in close contact with the laboratory for a long time or of course the joint paper for the first time with Prof. Dr. W. E. El-Saadawi. Paper from Dr. W. Krutzsch is extremely welcomed in every respect. He is really a famous scientist of the world in the Palynology. His activity in the taxonomy, palaeophytogeographical regions of the fossil sporomorphs, in palaeoclimatology and environmental evolution is fundamental, and authoritative. Personal and scientific contacts started at the beginning of the sixteenth's years. When this laboratory was under formation Dr. Krutzsch visited this laboratory first as invited scientist of the J. A. University.

As a practical point it is necessary to emphasize, that all publications of the laboratory, including the monographs too (one appeared, furthers are in preparation) are exchange material not commercial. All colleagues or institutions will receive these papers against for his own publication. Under- and postgraduate students who have not publication will also receive these papers for its request, if they want.

Szeged, 25, February, 1992.

M. Kedves
head of the laboratory

1. ÜBER VERMEINTLICHE DIPTEROCARPACEEN-POLLEN IM JUNGTERTIÄR EURASIENS (EIN BEITRAG ZUM „WACKERSDORFENSIS"-POLLENTYP)

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Zusammenfassung

Entdeckungsgeschichte, Nomenklatur, Morphologie und botanische Stellung sowie zeitliche und räumliche Verteilung von *Dipterocarpacearumpollenites hidasensis* (Synonym: *Fupingopollenites wackersdorfensis*) werden untersucht und dargestellt. Wie in diesem, so auch in einem anderen Falle, liegen keine Vertreter der die tropischen Tieflandsgebiete besiedelnden *Dipterocarpaceen* vor, sondern Pollenfunde einer bisher botanisch unbekannten Sippe, deren jungtertiäres eurasiatisches Verbreitungsgebiet im nördlichen Grenzbereich der Mixed-Mesophytic-Forests anzunehmen ist. Die Sippe ist in Europa/Vorderasien bis (resp. und) Ostasien ab höheren Miozän, z.B. im mediterranen Raum, als Tertiärrelikt aufzufassen und erlischt hier wahrscheinlich im Laufe des Pleistozäns. Für Ostasien besteht durchaus die Wahrscheinlichkeit, daß sie hier bis zur Gegenwart überlebt hat; nur hat man bisher die botanische Identifikation noch nicht ermitteln können.

Schlüsselwörter: Palynologie, fossil, Jungtertiär, *Angiospermae, Dipterocarpaceae.*

Einführung

Der im Titel genannte Pollentyp wurde erstmalig von MEYER (1952, Diss.) und 1956 (Publikation) aus Wackersdorf/Oberpfalz, BRD abgebildet, ohne aber in seiner Eigenart erkannt worden zu sein. Eine Beschreibung resp. Determination erfolgte dabei nicht. Vom Verfasser konnte der gleiche Typ Ende der 50iger Jahre als eigenständige Form in Präparaten erfaßt werden, die ihm L. RÜFFLE zur Einsicht und weiteren Auswertung überließ (Material zu seiner Dissertations-Arbeit über die sarmatische Flora des Randecker Maares, Potsdam 1961, Veröffentl. Berlin 1963). Hier konnte dieser Pollentyp relativ häufig und in unterschiedlichsten Erhaltungszuständen und Einbettungslagen nachgewiesen werden. Er wurde dann in den folgenden Jahren an zahlreichen Stellen im Tertiär der ehemaligen DDR (N-N. Sachsen, Oberlausitz Niederlausitz, Ostbrandenburg; alles Miozän) nachgewiesen und unter der internen Bezeichnung „*Tricolporopollenites randeckensis*" in den Fossil-Tabellen resp. Listen vermerkt. Zu einer gültigen Publikation der Art und der weiteren Einzeldaten ist es aber seinerzeit und — aus diversen Gründen — auch bis heute noch nicht gekommen, so daß der Artname „*randeckensis*" natürlich illegitim geblieben ist.

1969 veröffentlichte E. NAGY in ihrer ersten größeren Miozän-Monographie

unter zwei ganz verschiedenen Bezeichnungen (nämlich als „*Dacrydiumites guillauminii* n. sp." und als „*Dipterocarpacearumpollenites hidasensis* n. g. n. sp.") Pollenobjekte, die und darauf hat 1980, S. 153 schon THIELE-PFEIFFER mit Recht aufmerksam gemacht, wahrscheinlich den gleichen in Rede stehenden Formenkreis beinhalten. Die Beschreibung für „*Dacrydiumites guillauminii*" ist völlig abwegig; sie zeigt, daß von der Autorin das zugrunde liegende Typusobjekt morphologisch nicht sachgemäß erkannt worden ist. Dabei ist die Bezeichnung nicht nur für die Gattung, sondern auch für die Art irreführend und dubios, da sie dabei den Artnamen einer rezenten *Dacrydiumart* (*D. guillauminii* BUCHHOLZ) als n. sp. mit einem fossilen Organgattungs-Begriff verband. Weder die Organgattungsdeutung noch die Arterkennung (incl. Beschreibung) sind richtig, wie die Neuuntersuchung des Originalobjektes aus der Bohrung Zengővárkony 59 [Probe 22/1 (31,1/100,8), 51,3—56 m, „Helvet", graue schluffige Tonmergel] ergab. Es handelt sich in der Tat, wie schon THIELE-PFEIFFER auf Grund der alten Abbildungen richtig vermutete, völlig zweifelsfrei um einen Vertreter des in Rede stehenden Formenkreises. Diese Form ist eigenartiger Weise in der zweiten Miozän-Monographie von E. NAGY (1985) nicht wieder mit erwähnt, obwohl die THIELE-PFEIFFERsche Arbeit von 1980 im Literaturverzeichnis von E. NAGY angeführt ist.

Auch die weiteren bei NAGY 1969 (und 1985) angeführten mehr oder weniger direkt mit *Dacrydium* in Verbindung gebrachten Pollenformen belegen keinesfalls diese südhemisphärische Koniferengattung für das Jungtertiär Europas. Es handelt sich z. T. um saccusreduzierte, respektive anderweitig abnorme *Pinus*pollen, z. T. um cedroide Formen. Das ist 1985, S. 144 wenigstens für „*Dacrydiumites" taxoidiformis* selbst schon berichtigt worden, gilt aber auch für die drei restlichen Arten (S. 151), wobei für „*Dacrydiumites elegans* NAGY 1985" sogar die Frage offen bleibt, ob hier nicht wiederum der „*wackersdorfensis-Typ*" vorliegt (Taf. 86, Fig. 1). Was die andere NAGY-Art „*Dipterocarpacearumpollenites hidasensis*" angeht, so liegt auch hier der gleiche Pollentyp vor, der dann von THIELE-PFEIFFER als *T. wackersdorfensis* beschrieben worden ist. Die Beschreibung bei NAGY ist zwar nicht völlig korrekt, aber noch akzeptabel; die botanische Deutung hingegen, die im gebildeten Pollengattungsnamen, entsprechend der Mode jener Zeit und der Autorin, zum Ausdruck gebracht worden ist, gehört ins „Reich der Phantasie". Vertreter der die tropischen Tieflandsländer besiedelnden *Dipterocarpaceen* liegen hier mit Sicherheit nicht vor, aber der Fossil-Name ist nun mal so gebildet und nicht mehr änderbar (vgl. *Compositoipollenites* R. POT., eine Gruppe von Pollen, die keinesfalls *Compositen,* sondern *Icacinaceen* beinhaltet!). Die botanische Stellung der Form ist nach wie vor ungeklärt (s. unten). Da die Art „*hidasensis*" der Generotyp von *Dipterocarpacearumpollenites* ist, ist auch keine nomenklatorische Abkoppelung mehr möglich. Die weitere 1969 u. 1985 bei NAGY aufgeführte Art „*D. spinosus*" muß dagegen irgendwo anders untergebracht werden, denn hier handelt es sich um einen völlig anders gebauten spinosen Pollentyp, der aber, nach den gegebenen Beschreibungen und Abbildungen, im einzelnen ebenfalls noch unklar ist und erst einmal morphologisch neu zu untersuchen wäre, ehe eine botanische Zuordnung zu ermitteln möglich werden könnte. Dazu vergleiche man die gleichsinnigen Bemerkungen von MULLER (1981, S. 36). Dort ist auch zu einem anderen Pollenvergleich mit *Dipterocarpaceen* Stellung genommen, der von ROCHE

und SCHULER (1976) stammt (*Retitricolpites dipterocarpaceoides,* Oligozän/Belgien — die gleiche Art ist übrigens auch in der ehem. DDR im Oligozän an mehreren Stellen nachgewiesen). Auch dieser botanische Vergleich ist als nicht zutreffend zu bezeichnen. Beide *Dipterocarpaceen*-Vergleiche haben aber sonst — außer daß es keine *Dipterocarpaceen* sind — nichts miteinander gemein.

In den 70er Jahren wird unsere in Rede stehende Form nur zweimal aus Europa/Vorderasien als „unbekannter *tricolporater* resp. *Angiospermenpollen*" in der Literatur erwähnt, und zwar aus dem Pliozän des westlichen Schwarzen Meeres von KORENEVA und KARTASHOVA (1978) und aus Tiefseebodensedimenten (Sapropeliten) des östlichen Mittelmeeres, Pliopleistozän (Villafranchium) und U/M-Pleistozän von ROSSIGNOL-STRICK (1973), die sie bereits als botanisch noch nicht näher bekanntes „Tertiärrelikt" betrachtet (sofern es sich nicht um Umlagerungsprodukte handeln sollte).

1980 erfolgte die erste exakte Beschreibung der Form als „*Tricolporopollenites wackersdorfensis*" durch THIELE-PFEIFFER, die eindeutige und gute Abbildungen bringt (Tagebau Oder II, Schwandorf/Wackersdorf, Oberpfalz, BRD). Hier ist auch die erste kritische Synonymliste veröffentlicht (S. 153).

Schon 1979 war aber eine bisher in der europäischen Literatur nichtbeachtete Abbildung des gleichen Typs aus dem koreanischen Miozän von TAKAHASHI und KIM erschienen, allerdings wieder unter einer nicht korrekten, abwegigen Beschreibung und botanischen Deutung als „*Cedripites sacculatus* n. sp. (Taf. 13, Fig. 37 u. 39, ? 38; ? oder non Taf. 14, Fig. 1). Und dann fand sich die Form, wohl weit verbreitet, im west-und ostchinesischen Jungtertiär, ZHU et al. (1985), SONG et al. (1985). Dies weist darauf hin, daß hier eine (? nur z. Z. noch disjunkt nachgewiesene) europäisch-ostasiatische Sippe vorliegt, der paläochorologisch im Jungtertiär ein Gürtel-Areal etwa am Nordrand der damaligen Mixed-Mesophytic-Forests eigen gewesen zu sein scheint. Aber auch den chinesischen Palynologen ist bisher eine exakte botanische Zuordnung noch nicht gelungen. Sie unterscheiden inzwischen drei Arten, nach G. W. LIU (1985, S. 152) nun unter einem eigenen Pollengattungsbegriff „*Fupingopollenites*" (mit dem Generotypus „*wackersdorfensis*"). Die beiden weiteren Arten sind *F. minor* (35—45 µm) und *F. crassus* (ca. 50 µm) (alle Tsaidam Basin, Qinghai-Provinz, VR China).

Ergebnisse und Beschprechung

Durch die Revision der ungarischen Fossilvorkommen hat nunmehr als der älteste gültige Namen *Dipterocarpacearumpollenites hidasensis* NAGY, emend. nov. zu gelten. In Europa läßt sich bisher nur eine Art erkennen. Ob die beiden weiteren chinesischen Arten voll berechtigt sind, ist vorerst nicht zu entscheiden. Beide Arten wären, wenn ihre Selbständigkeit berechtigt sein sollte, entsprechend zu *Dipterocarpacearumpollenites* zu kombinieren, das *Fupingopollenites* nunmehr als Junior-Synonym zu gelten hat.

Erganzung der Synonymliste von 1980:

1. Die beiden Fragezeichen zu den Zitaten von NAGY (1969) können gestrichen werden.

2. Hinzu kommen:

1958 bis ca. 1981: „*Tricolporopollenites randeckensis*" — illegitimer, interner Name in den Listen u. Fossil — verzeichnissen der ehemaligen DDR-Palynologen (zahlreiche Berichte und Datenunterlagen).

1979 *Cedripites sacculatus* n. sp. — TAKAHASHI und KIM: 31, Taf. 13, Fig. 37 u. 39, ? 38; ? oder non.: Taf. 14, Fig. 1 (evtl. Fragment *Pinus*) (alle Miozän, Korea).

1985 *Dipterocarpacearumpollenites hidasensis* NAGY 1969 — NAGY 1985: 183, Taf. 106, Fig. 1 (Hidas, unteres Mittel-Miozän, Badenian) non: Exemplar zu Tafel 105, Fig. 21 (Sarmat, Ungarn) (Das im Text genannte Objekt aus dem Oberpannon Ungarns ist nicht abgebildet und bleibt deshalb vorerst unklar).

1985 *Fupingopollenites wackersdorfensis* (TH. et PF.) G. W. LIU 1985
F. minor SONG und ZHU ?
F. crassus SONG und ZHU } eigene Arten?
alle aus ZHU et al. 1985: 209, Taf. 58
(Tsaidam-Becken, VR China, Mittel- bis Jungtertiär).

1985 *Fupingopollenites* nov. gen. (G. W. LIU) — in SONG et al. 1985: 152, (Longjing Area Shelf, Ostchinesiches Meer, Jungtertiär).

Emendierte Gattungsdiagnose von *Dipterocarpacearumpollenites:*

Tricolporat, Wand komplex-reticulat-columellat, Oberfläche außerhalb Colpensektoren mit bis zu 8 muldenförmig eingesenkten rundlichen Feldern, in denen die Wand und damit auch die Struktur sehr reduziert ist. Germinale und Cavernen sehr zart.

Durch die Differenzierung der extracolpaten Oberfläche und der Wandbauverhältnisse von allen anderen tricolporaten Pollengenera unterschieden. Verfaltete und schlecht erhaltene Objekte sind in der Vergangenheit oft mit saccusreduzierten *Coniferen*-Pollen verwechselt worden.

Emendierte Artdiagnose von *D. hidasensis:*

Relativ große tricolporate Pollen von annähernd kugeliger Figura mit vieleckigen Aufsichtkonturen. Nur bei reinen Pollagen finden sich eck-abgestutzte dreieckige Konturen, wobei die Colpen im Äquatorbereich meist klaffen. Colpen relativ kurz (etwa 1/2 bis 2/3 r), zart, da Wand in Richtung auf Colpen stark verjüngen. Poren rundlich, z. T. längs oder breit oval verzogen, Cavernen sehr zart, daher in der Regel kaum zu sehen. Oberfläche außerhalb der Colpen-sektoren in maximal 8 gleichgroße muldenartig eingesenkte rundliche Felder aufgeteilt. Wand besonders in den Randzonen der Mulden komplex columellat-reticulat. Wandstärke von 3 µm zwischen den Mulden bis auf 1 µm in den Mulden zurückgehend; dann Stäbchenelemente entsprechend zarter und kürzer.

Chorologie und Stratigraphie:

Mitteleuropa nördlich der Alpen und Ungarn bisher Untermiozän bis höheres Mittelmiozän (Sarmat). Es bleibt offen, ob hier durchgehend oder nur vikariierend in einzelnen Abschnitten vertreten. Südliches und südöstliches Europa bis Vorder-

asien: Bisher nur im Pliozän bis? Mittelpleistozän. Ostasien: Mittel- bis Jungtertiär, West- und Ostchina, Korea. Erst mit weiteren Fossilnachweisen wird die genauere Zeit-Raum-Verbreitung zu ermitteln sein. Sicher ist aber schon jetzt, daß es sich auch nach der paläo-chorologischen Situation und dem stratigraphischen Auftreten keinesfalls um Vertreter der auf die Tieflandtropen beschränkten *Dipterocarpaceen* handeln kann, wie die vorschnelle und sehr unglückliche, aber nicht mehr zu verändernde Namensbildung suggerieren könnte. Bei *Dipterocarpacearumpollenites* handelt es sich nach den bisherigen Fossildaten um eine Pflanzensippe, die wahrscheinlich im nördlichen Grenzbereich der Mixed-Mesophytic-Forests, möglicherweise auch in Übergangsbereichen mit gewissen xerothermen Einschlägen, anzusiedeln ist. Sie hat möglicherweise im ostasiatischen Raum irgendwo noch bis heute überlebt, während sie in Europa/Vorderasien, spätestens seit dem Ende des Miozäns bis ins Quartär hin, als Relikt anzusprechen und mit großer Wahrscheinlichkeit hier sukkzesive erloschen ist (ROSSIGNOL-STRICK 1973, THIELE-PFEIFFER 1980).

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2. SOBRE EL CARACTER INTERDISCIPLINAR DE LOS ESTUDIOS PALEOBOTÁNICOS

Interdisciplinary character of the palaeobotanical studies

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El fundamento de la Paleontología es esencialmente biológico, basado en la biodiversidad y la evolución y como tal está intimamente ligada con la Bioquimica y la Biofisica.

El rasgo diferencial estriba en que los seres que se estudian vivieron en otras épocas geológicas y sus restos aparecen en los sedimentos formando unas asociaciones distintas de las originarias con unas implicaciones geológicas innegables.

Los fósiles no constituyen unas individualidades a estudiar separadamente del entorno en que vivieron pués formaban parte de asociaciones que habitaban ecosistemas componiendo un todo dificil de desligar, que debe ser estudiado en su conjunto, además de tener en cuenta las alteraciones que posteriormente pudieron sufrir estos depósitos.

En el caso concreto de los estudios paleobotánicos, estos abordan no solamente la descripción anatómica y morfológica, atribución taxonómica y distribución estratigráfica de los restos vegetales sino que pueden abarcar una serie de factores que influyen muy directamente en los procesos tafonómicos como son la abundancia, caso de los restos foliares y palinomorfos en relación a los ejes, tallos, flores etc., la dispersión y la fosilización selectiva.

El análisis de los diversos factores geológicos, quimicos y fisicos que influyen más o menos directamente en esta fosilización, como pueden ser la resistencia a la degradación de cada una de las sustancias que componen las diferentes partes del ser vivo que puede comenzar incluso antes de la muerte, los procesos postmortem a que va a estar expuesto etc. nos aportarán datos de gran valor para que junto con los estudios morfológicos, histológicos, bioquimicos y evolutivos obtengamos una visión lo más completa posible acerca de esos restos de la flora de otros periodos geológicos que ha llegado hasta nosotros.

En base a esta multidisciplinaridad, la cooperación entre grupos de trabajo con formación diversa e instrumental complementario permite un mejor aprovechamiento de los recursos humanos y materiales con vistas a una competitividad en las investigaciones.

De ahí se desprende lo beneficioso de los intercambios entre investigadores de diversos paises con inquietudes similares para un mejor conocimiento entre ellos y una más estrecha relación que redunde en una investigación más acorde con los tiempos y más universalista, pues incluso problemas paleobotánicos de interés local o regional pueden ser resueltos en base a la comparación con otros similares de otras áreas geográficas o periodos geológicos.

En el caso concreto que nos ocupa, la cooperación hispano-húngara en relación a temas paleobotánicos y en particular palinológicos comienza en 1962, cuando uno de los autores (M. KEDVES) y la Dra. N. SOLÉ DE PORTA, en aquellos años en Colombia, abordaron el estudio comparativo de las esporas del género *Cicatricosisporites* de Hungría y Colombia, poniendo de manifiesto algunas similitudes muy interesantes entre ellas.

Con posterioridad esas colaboraciones continuaron tanto con la Dra. N. SOLÉ como con los Profesores J. PORTA y J. CIVIS de las Universidades de Barcelona y Salamanca, publicando dos artículos sobre los palinomorfos del Cretácico superior del Barranco de la Posa.

Destaca así mismo que desde 1982 M. KEDVES ha participado en los Simposios que bianualmente organiza la Asociación de Palinológos de Lengua española (APLE) donde presenta sus avances en el estudio de la degradación de la pared esporopolínica por métodos experiementales, así como sobre la composición de la misma.

Las estancias en España de M. KEDVES han sido numerosas, tanto para realizar trabajos como para asistir a Congresos. En la II Conferencia Europea de Paleobotánica celebrada en Madrid en 1989 pronunció la conferencia inagural sobre "New trends in Micropaleontological researches".

Hay que destacar que en 1986 la Universidad de Salamanca le concedió la *"Medalla universitaria con el sello del estudio que se entrega en conmemoraciones o en atención a servicios distinguidos"*.

Todas estas actividades se realizaron a titulo más o menos personal y no enmarcadas en las relaciones institucionales entre los organismos de investigación de España y Hungría.

Por ello en la actualidad se están realizando los contactos necesarios, con visitas a los centros implicados, para la elaboración de un proyecto de investigación hispano-húngaro bajo los auspicios del CSIC y la Academia de Ciencias de Hungría en el que se abordarían estudios palinológicos entre el personal dela U. E. I./Dep. de Paleontología del Instituto de Geología Económica de Madrid (Prof. ALVAREZ RAMIS y uno de los autores de esta nota M. T. FERNÁNDEZ MARRÓN) y el Laboratorio de Biología celular y Micropaleontologia evolutiva (el otro autor M. KEDVES) del Departamento de Botánica de la Universidad J. A. de Szeged.

Sería deseable que este proyecto recibiese la aprobación de los comites competentes de selección para que la colaboración entre ambos paises en el campo de la Paleobotánica tan fructífera anteriormente, se incremente y contribuya a un mayor desarrollo de la Ciencia.

Después de la elaboración de la presente nota la Comisión Mixta de Cooperación Cientifico-Técnica entre España y Hungría del Ministerio de Asuntos exteriores aprobó el proyecto de investigación conjunta „Estudio de diversos

aspectos paleobotánicos del Cretácico superior del Cerro de la Mesa, Madrid." para 1992—93.

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3. HIGH TEMPERATURE EFFECT OF SOME BISACCATE GYMNOSPERM POLLEN GRAINS

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Abstract

The qualitative and quantitative secondary alterations of the pollen grains of *Pinus silvestris*, *Pinus mugo* and *Picea glauca* were investigated. The pollen grains were heated at 200 °C during different lengths of time. Peculiar attention was payed to the nomenclature of the light microscopical morphology of the bisaccate pollen grains. The high temperature effect damages the sacci. After a long length of time heating the pollen grains lost the bladders, and these secondary altered forms are similar to early sulcate fossil taxa, such as *Bennettitaceaeacuminella*, etc. The alterations in the size are evaluated by the corpus and the saccus.

Key words: Palynology, recent, bisaccate *Gymnospermatophyta*, high temperature effect.

Introduction

It was emphasized previously that after our first observations on the secondary alterations of the *Brevaxonate Amentiflorae* pollen grains (KEDVES and KINCSEK, 1989) in consequence of the high temperature effect, several research programs started with different basic concepts. The LM and the TEM method was used. These two methods have very different aspects. The LM method is important in the investigations of the fossil spores and pollen grains, in particular at those extracted from the metamorphic layers. The secondary alterations of the saccate gymnosperm pollen grains are included in the systematic study of all kinds of spore and pollen types. In this way the aims of this research are as follows.

i. To establish the qualitative morphological alterations in particular which has taxonomic importance.
ii. To investigate the secondary alterations of the size of the corpus and the saccus.
iii. Interpretation of the experimental data with the fossil types from different geological ages.

Material and Methods

The investigated species are the following:

Pinus silvestris L.

Collected: Dr. K. Margóczi, 18. 5. 1988., in the Botanical Garden of the J. A. University. Experiment numbers: 246–350, 905, 907, 910, 759, 913, 916.

Pinus mugo Turra

Collected: Dr. K. Margóczi, 18. 5. 1988., in the Botanical Garden of the J. A. University. Experiment numbers: 366–370, 445, 908, 911, 760, 914, 917.

Picea glauca (Moench.) Voss.

Collected: Dr. K. Margóczi, 18. 5. 1988., in the Botanical Garden of the J. A. University. Experiment numbers: 351–355, 906, 909, 912, 758, 915, 918.

Temperature: 200 °C.

Length of time:
1h Experiment No: 346, 366, 351
2h Experiment No: 347, 367, 352
3h Experiment No: 348, 368, 353
4h Experiment No: 349, 369, 354
5h Experiment No: 350, 370, 355
10h Experiment No: 905, 445, 906
25h Experiment No: 907, 908, 909
50h Experiment No: 910, 911, 912
100h Experiment No: 759, 760, 758
200h Experiment No: 913, 914, 915
300h Experiment No: 916, 917, 918

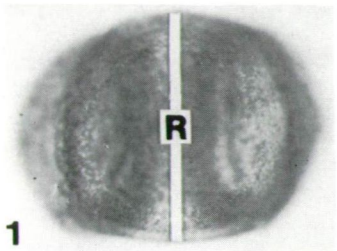
The pollen grains were mounted in glycerin-jelly hydrated at 39.6%, and investigated with the light-microscope method.

As regards the light microscopical morphology of the bisaccate gymnosperm pollen grains there are several concepts, in particular the points of symmetries and the measurements of the different parts of the pollen grain. There are a lot of publications in this subject, some selected establishments are summarized as follows.

Aytug (1959) used the data of the quantitative measurements to establish the hybride character of *Abies Equi Trojani* Aschers et Sinten. Basic establishments were published later (Aytug, 1960). By the repeated measurements of the same slide in different intervals (one, two and four months) the expansion of the corpus was observed. Worth of mentioning is the fact that the dimension of the saccus has not altered. Later (Aytug, 1962) measured the parameters of four species of the genus *Cedrus* Link. Barth (1962) used also the data of the measurements of the corpus, saccus and the total diameter, in two positions of the pollen grain. Freudenthal (1964) in his paper emphasized as follows., p. 213: "Jansonius (1962) was the first to give a good method of describing of bisaccate pollen grains." Krutzsch (1971) reviewed several previous publications in this respect, in connection with the monograph of the Middle and Upper Tertiary fossil gymnosperm pollen grains. Sivak (1975) dealt also in detail with this problem, and pointed out the following, p. 352: "Krutzsch (1971) a fait une synthèse partielle des termes utilisés; nous regrettons qu'il n' existe pas de vocabulaire commun, voici celui que nous proposons (fig. 1):" The proposal of Sivak (1975) was slightly modified, and it is presented in Plate 3. 1., with the original French terms. Accorsi et al. (1978) investigated from this point of view the species of the genus *Pinus* from Italy. As regards the general morphology of these pollen grains we cite from the publication of Lieux (1980), p. 20, 21: "Type 2" "Pollen description: disaccate, heteropolar, monosulcate. Sulcus represented by a thinned area on distal side of grain; sulcus area more or less psilate in all species examined." Pocknall (1981) investigated in detail the New Zealand pollen grains of the species of *Dacrydium* Solander, *Podocarpus* L'Héritier and *Dacrycarpus* Endlicher. Basic morphological establishments were published. But it is necessary to emphasize that the term saccus "reticulum" was used inside the alveoli. Riabkova (1982) dealt in detail with the LM and TEM structure of the gymnosperm pollen grains. Further important data are in the paper of Hansen and Engstrom (1985) and Brady (1988).

Plate 3. 2. summarizes the basic, general morphological establishments about the bisaccate gymnosperm pollen grains. For the saccus alveolar structure the paper of M. Van Campo (1973) was used. In general the above mentioned and discussed works were followed with attention. The 16 (extremal) landmarks are from the publication of Brady (1988), p. 487:

VUE PROXIMALE



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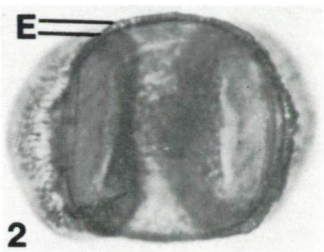
épaisseur de la calotte E

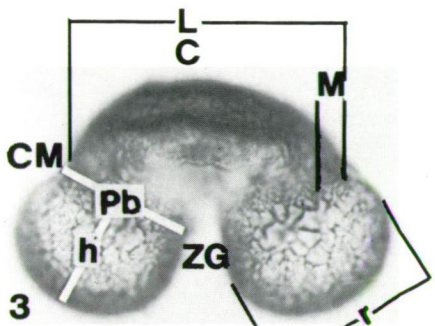
R= largeur du corps



2

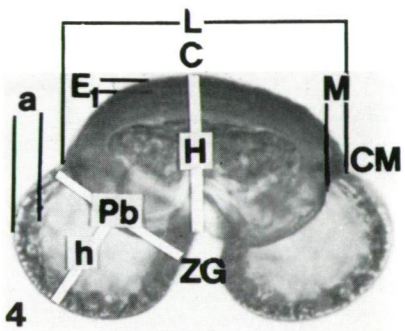
VUE DE PROFIL

L=longueur du corps
H=hauteur du corps



3

M=épaisseur de la crête marginale



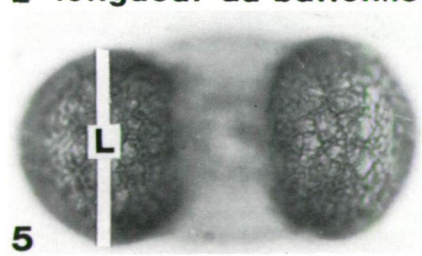
4

C=calotte
ZG=zone germinale
CM=crête marginale
Pb=plancher du ballonnet

E_1=épaisseur de la calotte
h=hauteur du ballonnet
r=largeur du ballonnet
a=hauteur des alvéoles

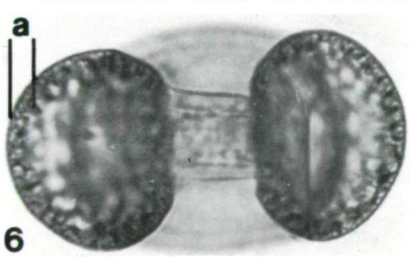
VUE DISTALE

L=longueur du ballonnet



5

hauteur des alvéoles



6

◀ Plate 3.1.

Pinus mugo TURRA

Fresh pollen grains coloured with Toluidin blue, from different aspects. The morphological terms follows the paper of SIVAK (1975). 1000 x.

"a Proximal pole Proximal-most point on pollen grain; bisector of arc of corpus between proximal radices.
b Distal pole Bisector of arc of corpus between distal radices, or if distal groove present, then point at base of groove.
c Right proximal radix Proximal-most point of attachment of right saccus to corpus.
d Left proximal radix Proximal-most point of attachment of left saccus to corpus.
e Right terminus Right-most point on pollen grain; point furthest to right of line between proximal and distal poles.
f Left terminus Left-most point on pollen grain; point furthest to left of line between proximal and distal poles.
g Left centrum Bisector of line between proximal and distal radices of left saccus.
h Left vertex Bisector of arc between proximal and distal radices of left saccus.
i Left distal flexus Distal-most point on line perpendicular to, and bisecting, line between centrum and vertex of left saccus.
j Left proximal flexus Proximal-most, point on line perpendicular to, and bisecting, line between centrum and vertex of left saccus.
k Right distal radix Distal-most point of attachment of right saccus to corpus.
l Left distal radix Distal-most point of attachment of left saccus to corpus.
m Right centrum Bisector of line between proximal and distal radices of right saccus.
n Right vertex Bisector of arc between proximal and distal redices of right saccus.
o Right distal flexus Distal-most point on line perpendicular to, and bisecting, line between centrum and vertex of right saccus.
p Right proximal flexus Proximal-most point on line perpendicular to, and bisecting, line between centrum and vertex of right saccus."

The enumerated publications in the following are also important from the point of view of the bisaccate gymnosperm pollen grains: VAN CAMPO-DUPLAN (1946, 1947 a, b), M. VAN CAMPO (1973), M. VAN CAMPO and SIVAK (1972), MARTENS and WATERKEYN (1961), VASIL (1978), and KURMANN (1989).

To get quantitative data for the degradation of the sacci, three groups were established (Text-fig. 3. 1.):

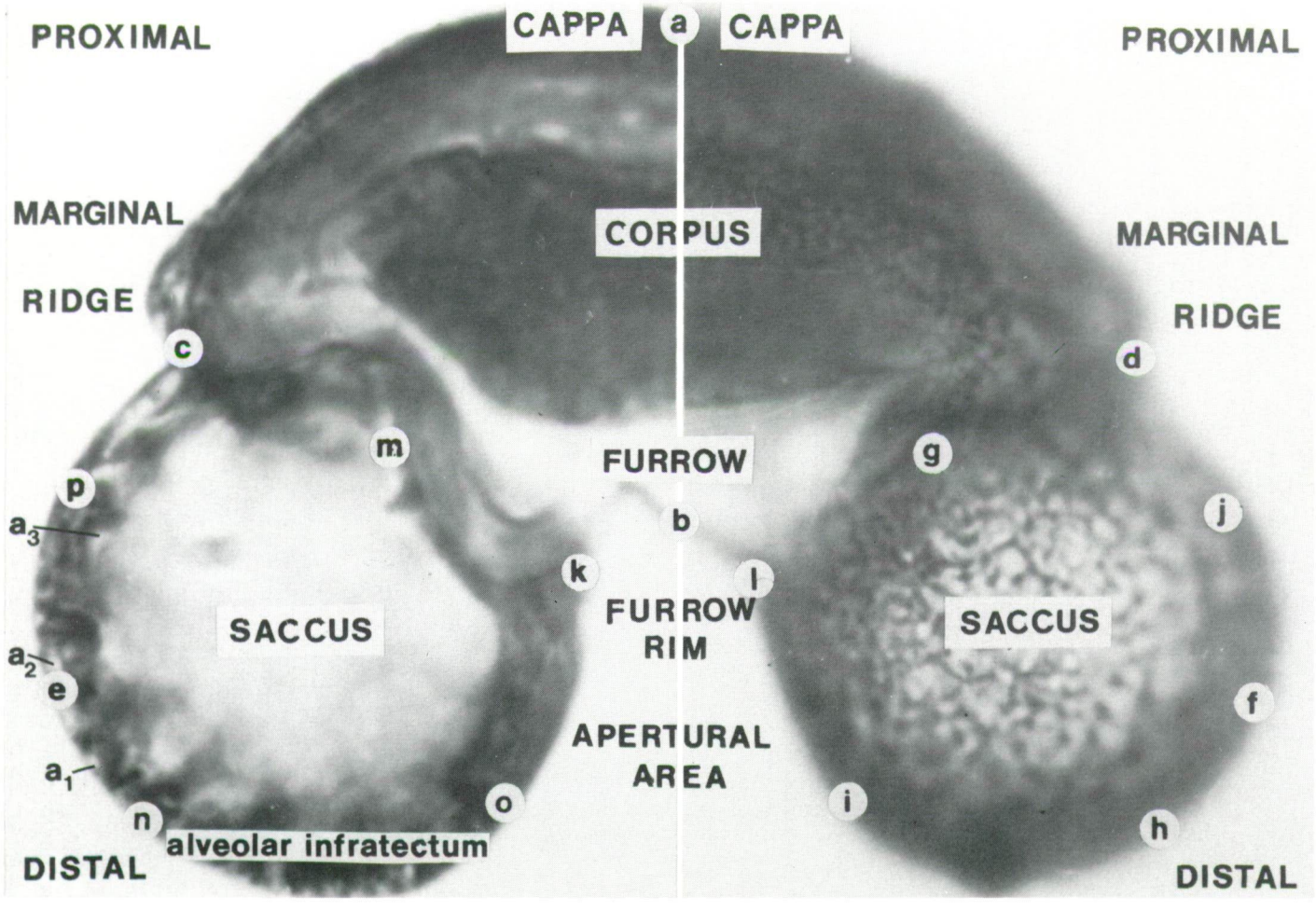
i. The intact form with two sacci (bladders).
ii. The partially degraded pollen grains, in general with one saccus.
iii. The corpus, without saccus.

For the quantitative investigations of the bisaccate forms, the following parameters were measured (Plate 3. 2.). Corpus: c—d (corpus breadth), and a—b (corpus height). Saccus: p—o (saccus width) and g—h (saccus height). The alterations in consequence of the high temperature of the pollen grains are illustrated in Plate 3. 3.—3. 6.

Plate 3.2. ▶

Pinus mugo TURRA

Fresh pollen grain coloured with Toluidin blue. The surface and the optical sections are illustrated, with the most important terms in English, and points of symmetries. Particularly the following publications were used: M. VAN CAMPO (1973), POCKNALL (1981), HANSEN and ENGSTRÖM (1985) and BRADY (1988).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CAPPA
PROXIMAL
MARGINAL
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CORPUS
MARGINAL
RIDGE
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a3
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l
FURROW
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SACCUS
SACCUS
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APERTURAL
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alveolar infratectum
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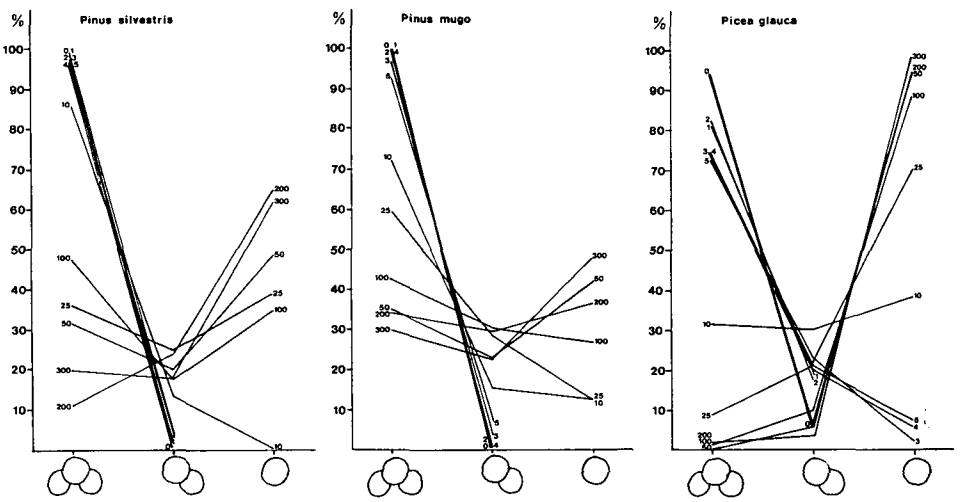
Results

Pinus silvestris L.
(Plate 3.3., figs. 1—51, text-fig. 3.1., 3.2., and 3.3)



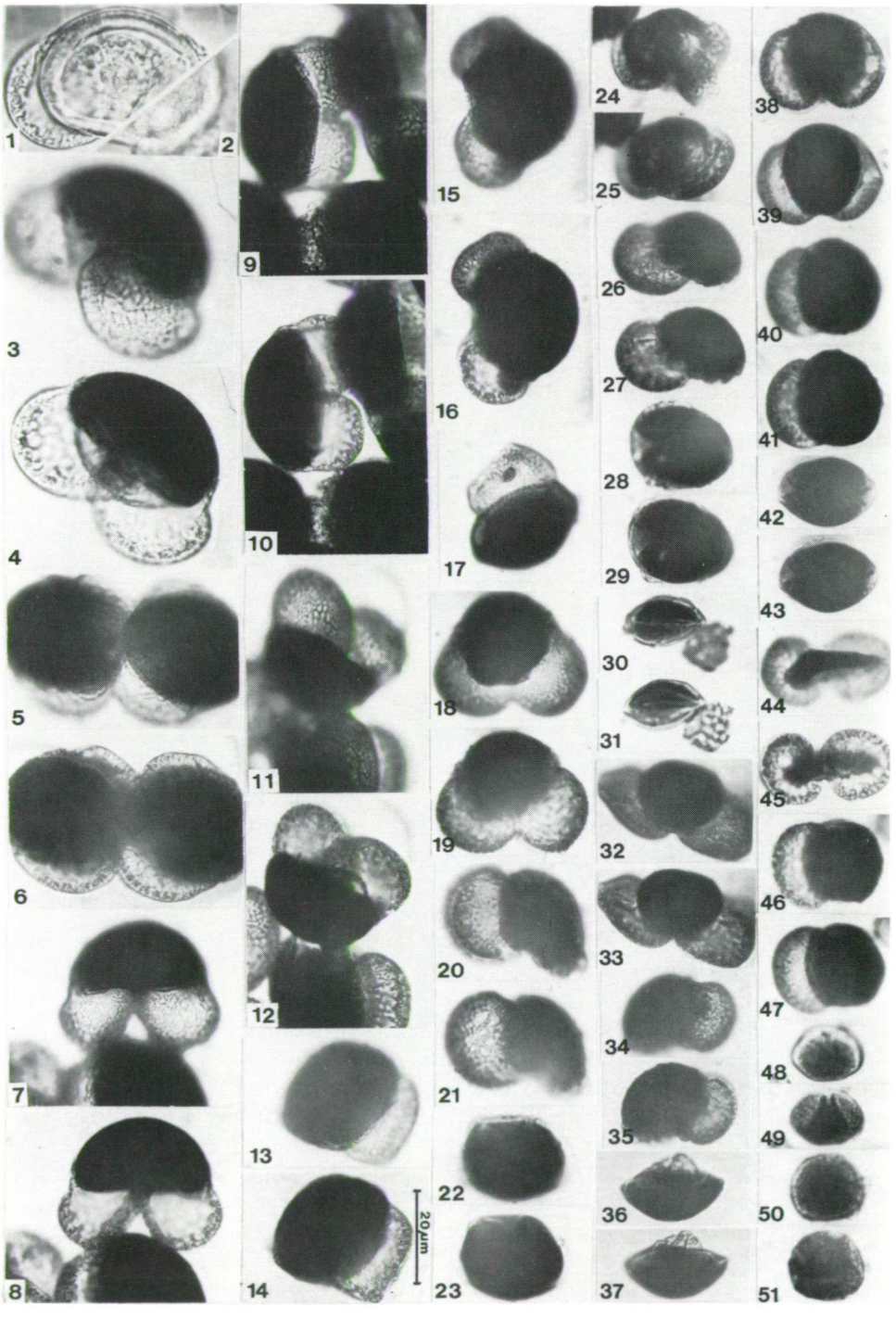
Text-fig. 3.1.

Variation statistical graphs of the saccus degradation of the species investigated, in consequence of high temperature (200 °C). The numbers at the graphs indicate the length of times of heating.

The microphotographs in Plate 3.3 well illustrate the alterations, which appeared in consequence of the high temperature. The pollen grains without saccus can be similar to extremely altered algal cysts (Plate 3.3., fig. 22, 23), or fossil monosulcate form genera, such as *Bennettitaceaeacuminella* MALYAVKINA 1953 (Plate 3.3., fig. 30, 31), or *Gynkgaletes* LYUBER 1955 (Plate 3.5., fig. 42, 43) (cf. POTONIÉ, 1958).

In connection with the saccus degradation the results of the non-experimental and those heated during 1—5 hours are essentially the same. The bisaccate forms are predominant, with very few monosaccate pollen grains. The small differences between the above mentioned experiments are well-regulated. The first important change appears after the heating during 10 hours. Remarkable (more than 10 per cent) is the quantity of the monosaccate forms, and the pollen grains without saccus appear. The graphs representing the degradation process from 25—300 hours show another group, which is characteristically different from the above discussed ones. In general, the quantities of the disaccate forms diminish, and the per cents of the non-saccate pollen grains increase. But it is interesting that these alterations are irregular. The most important "anomaly" can be established at the pollen grains heated during 100 hours.

The alterations in the dimension of the corpus can be summarized as follows (Text-fig. 3.2.).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20µm

◀ Plate 3.3.

1—51. *Pinus silvestris* L., Recent.
1, 2. Pollen grains without staining or heating.
3, 4. Experiment No 346, length of time 1 hr.
5, 6. Experiment No 347, length of time 2 hrs.
7, 8. Experiment No 348, length ot time 3 hrs.
9, 10. Experiment No 349, length of time 4 hrs.
11, 12. Experiment No 350, length of time 5 hrs.
13, 14. Experiment No 350, length of time 5 hrs.
15, 16. Experiment No 905, length of time 10 hrs.
17. Experiment No 905, length of time 10 hrs.
18, 19. Experiment No 907, length of time 25 hrs.
20, 21. Experiment No 907, length of time 25 hrs.
22, 23. Experiment No 907, length of time 25 hrs.
24, 25. Experiment No 910, length of time 50 hrs.
26, 27. Experiment No 910, length of time 50 hrs.
28, 29. Experiment No 910, length of time 50 hrs.
30, 31. Experiment No 910, length of time 50 hrs.
32, 33. Experiment No 759, length of time 100 hrs.
34, 35. Experiment No 759, length of time 100 hrs.
36, 37. Experiment No 759, length of time 100 hrs.
38, 39. Experiment No 913, length of time 200 hrs.
40, 41. Experiment No 913, length of time 200 hrs.
42, 43. Experiment No 913, length of time 200 hrs.
44, 45. Experiment No 916, length of time 300 hrs.
46, 47. Experiment No 916, length of time 300 hrs.
48, 49. Experiment No 916, length of time 300 hrs.
50, 51. Experiment No 916, length of time 300 hrs.

Corpus breath

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0^h	40.0	47.5	55.0	15.0 μm
1^h	35.0	40.0	50.0	15.0 μm
2^h	27.5	37.5	42.5	15.0 μm
3^h	32.5	37.5	42.5	10.0 μm
4^h	30.0	37.5	45.0	15.0 μm
5^h	27.5	35.0	42.5	15.0 μm
10^h	27.5	35.0	52.5	25.0 μm
25^h	22.5	30.0	37.5	15.0 μm
50^h	22.5	27.5	32.5	10.0 μm
100^h	22.5	25.0	30.0	7.5 μm
200^h	20.0	25.0	30.0	10.5 μm
300^h	17.5	22.5	27.5	10.0 μm

Regarding these data, the following can be emphasized.

Similarly to the saccus degradation, two groups of graphs can be distinguished based on the length of times in hours at 200 °C: 1—5 and 25—300. The length of time of heating during 10 hours in also peculiar, particularly the value of the distance of the smallest and largest specimens.

The so-called dislocation of the graphs is nearly at the heating during 25 hours. (The largest pollen grain of the experimental sample is identical or near identical with the smallest specimen of the fresh — non-experimental — material).

Corpus height

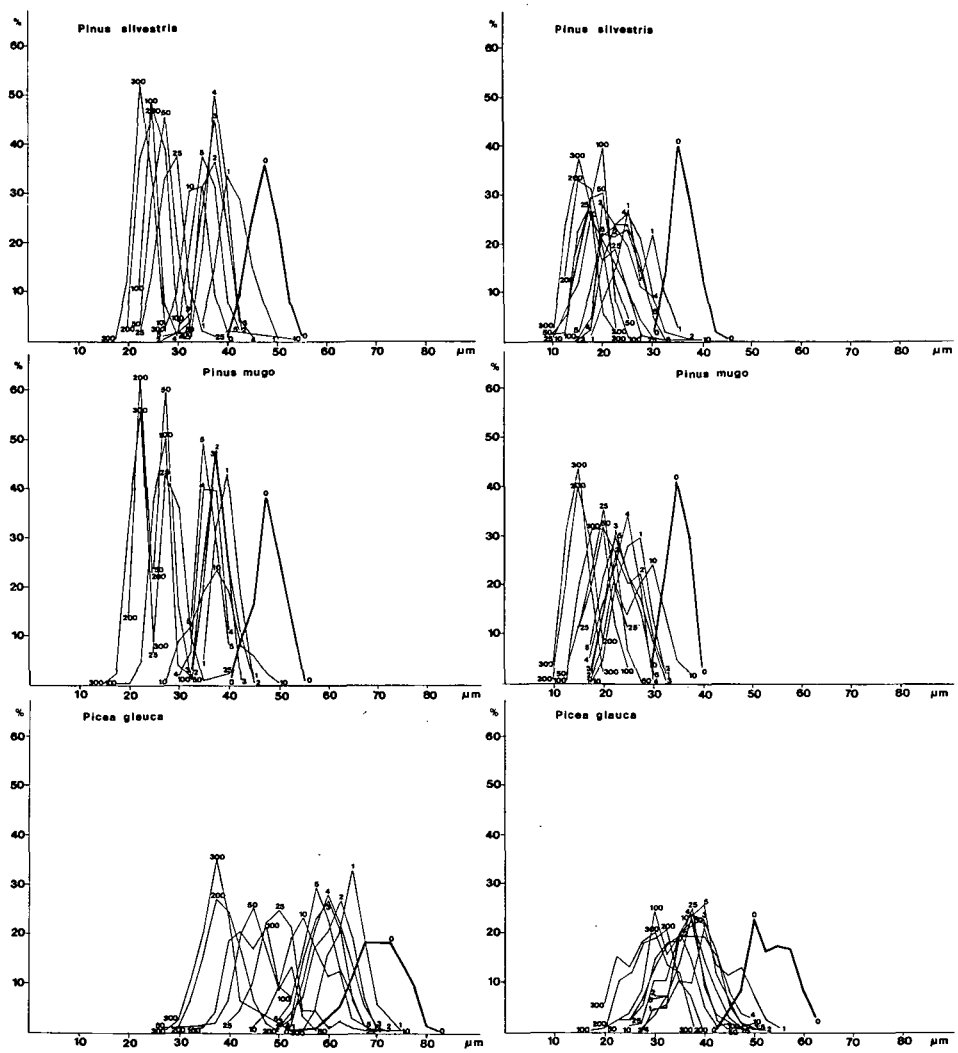
Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	30.0	35.0	45.0	15.0 μm
1h	17.5	25.0	35.0	17.5 μm
2h	15.0	22.5	37.5	22.5 μm
3h	15.0	20.0	32.5	17.5 μm
4h	17.5	25.0	30.0	12.5 μm
5h	15.0	25.0	30.0	15.0 μm
10h	10.0	17.5	37.5	27.5 μm
25h	10.0	17.5	30.0	20.0 μm
50h	10.0	20.0	25.0	15.0 μm
100h	12.5	20.0	25.0	12.5 μm
200h	12.5	15.0	22.5	10.0 μm
300h	10.0	15.0	22.5	12.5 μm

The two groups of the graphs of the experimental material can also be established. But these two groups separate not so well as previously, at the corpus length. There are differences, as follows. The graph of the heating during one hour have two maxima. At the maxima of the graphs, there are some "anomalies" in contrast to the previous. So, the lengths of times of one hour 2 and 4 hours represent one group. The maxima of the further experiments (3, 5, 10, 25, 100, 200, 300) are in another group. There is not so characteristic difference of the experimental data of the pollen grains heated during 200 and 300 hours. As regards the distances in μm between the smallest and the largest specimens, this is in general 12.5—17.5 μm. Extreme values were observed at 2, 10 and 25 hours, in particular at 10 hours.

The dislocation of the non-experimental, and the experimental graph is exactly at 25 hours of heating.

Saccus width

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	22.5	32.5	40.0	17.5 μm
1h	17.5	27.5	35.0	17.5 μm
2h	17.5	25.0	30.0	12.5 μm
3h	17.5	22.5	27.5	10.0 μm
4h	20.0	22.5	30.0	10.0 μm
5h	17.5	25.0	32.5	15.0 μm
10h	17.5	22.5	32.5	15.0 μm
25h	15.0	20.0	30.0	15.0 μm
50h	15.0	20.0	25.0	10.0 μm
100h	15.0	20.0	25.0	10.0 μm
200h	12.5	17.5	22.5	10.0 μm
300h	12.5	17.5	20.0	7.5 μm

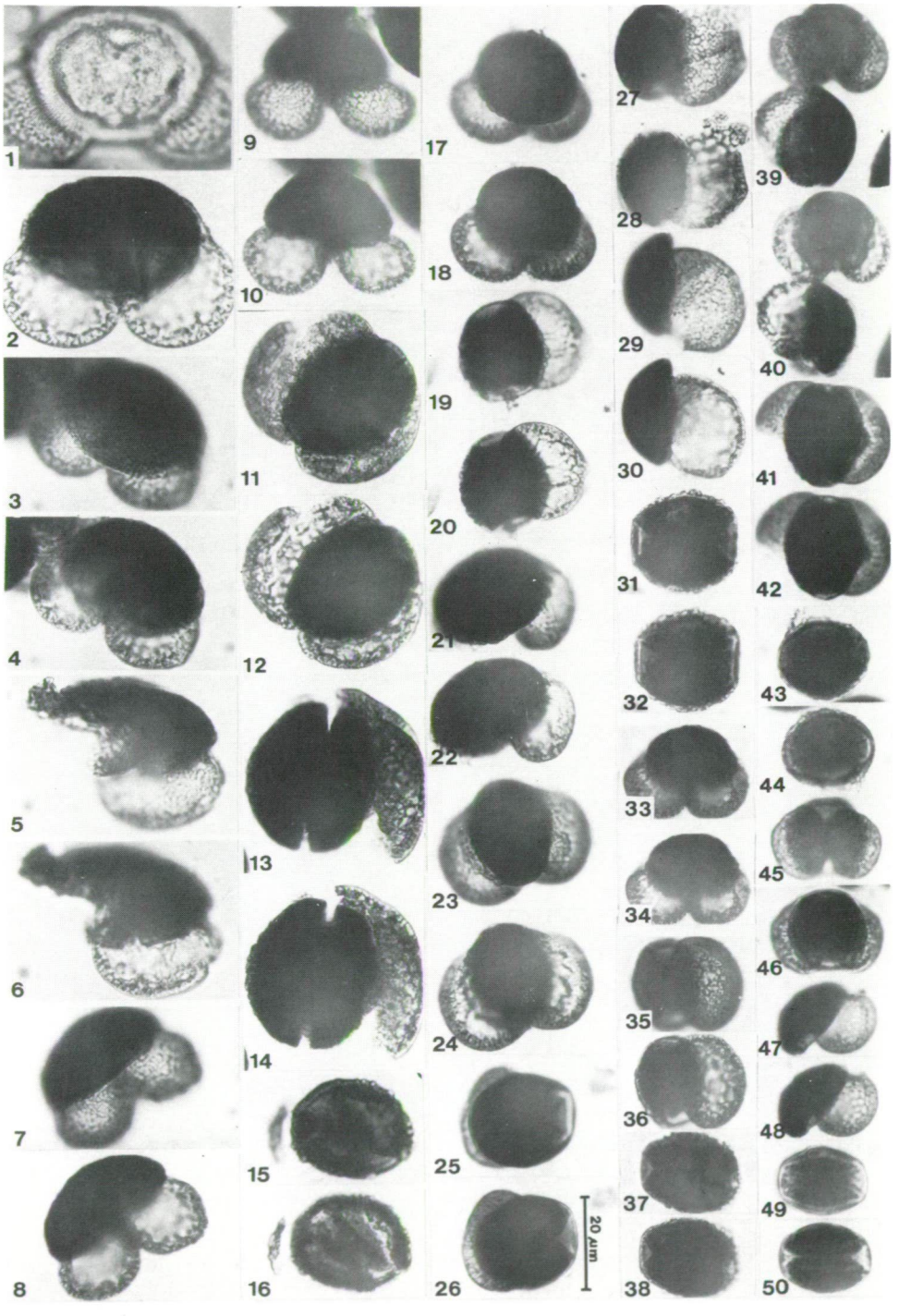


Text-fig. 3.2.

Variation-statistical graphs of the alterations of the corpus breadth and height in consequence of high temperature. The numbers at the graphs indicate the length of times of heating at 200 °C.

The graphs of the widths of the saccus after experiment are in one group only, in contrast to the corpus. The non-experimental graph is slightly separated from the others. There is an approximatively gradual alteration, which is not completely regular. Pairs worth of mentioning can be established in the maxima of the graphs of experiments 50 and 100 hours, respectively 200 and 300 hours.

The dislocation of the non-experimental and experimental graph is exactly at 200 hours of heating.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◀ Plate 3.4.

1—50. *Pinus mugo* TURRA, Recent.
1. Pollen grains without staining or heating.
2. Experiment No 366, length of time 1 hr.
3, 4. Experiment No 367, length of time 2 hrs.
5, 6. Experiment No 368, length of time 3 hrs.
7, 8. Experiment No 369, length ot time 4 hrs.
9, 10. Experiment No 370, length of time 5 hrs.
11, 12. Experiment No 445, length of time 10 hrs.
13, 14. Experiment No 445, length of time 10 hrs.
15, 16. Experiment No 445, length of time 10 hrs.
17, 18. Experiment No 908, length of time 25 hrs.
19, 20. Experiment No 908, length of time 25 hrs.
21, 22. Experiment No 908, length of time 25 hrs.
23, 24. Experiment No 911, length of time 50 hrs.
25, 26. Experiment No 911, length of time 50 hrs.
27, 28. Experiment No 911, length of time 50 hrs.
29, 30. Experiment No 911, length of time 50 hrs.
31, 32. Experiment No 911, length of time 50 hrs.
33, 34. Experiment No 760, length of time 100 hrs.
35, 36. Experiment No 760, length of time 100 hrs.
37, 38. Experiment No 760, length of time 100 hrs.
39, 40. Experiment No 914, length of time 200 hrs.
41, 42. Experiment No 914, length of time 200 hrs.
43, 44. Experiment No 914, length of time 200 hrs.
45, 46. Experiment No 917, length of time 300 hrs.
47, 48. Experiment No 917, length of time 300 hrs.
49, 50. Experiment No 917, length of time 300 hrs.

The distance between the maximum and minimum values vary from 17.5 μm until 7.5 μm. A not completely regular decreasing may be established at these values. Extremely sudden values have not been observed.

Saccus height

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	15.0	22.5	30.0	15.0 μm
1h	10.0	15.0	27.5	17.5 μm
2h	10.0	12.5	20.0	10.0 μm
3h	7.5	12.5	17.5	10.0 μm
4h	10.0	12.5	17.5	7.5 μm
5h	7.5	12.5	27.5	20.0 μm
10h	10.0	15.0	22.5	12.5 μm
25h	7.5	12.5	20.0	12.5 μm
50h	7.5	12.5	20.0	12.5 μm
100h	7.5	10.0	17.5	10.0 μm
200h	7.5	10.0	15.0	7.5 μm
300h	7.5	10.0	15.0	7.5 μm

All experimental variation-statistical graphs represent one group. This separates quite well from the non-experimental one. Taking into consideration the fine details, the following can be pointed out. The maxima of the experiments of one hour and 10 hours are at the same value. The maxima of 25, 5, 2, 3, 50 and 4 hours represent another group. Finally the maxima of the heated pollen grains during 100, 300 and 200 hours, represent another group. The irregular decreasing in the height of the saccus is extremely expressive.

Similarly to the saccus width, the dislocation of the non-experimental, and experimental graphs is exactly at heating during 200 hours.

The distances between the maximum and minimum values vary from 20.0—7.5 μm. As extreme value, the 20.0 μm can be pointed out at 5 hours of heating.

Pinus mugo TURRA

(Plate 3.2., and 3.4., figs. 1—50, text-fig. 3.1., 3.2., and 3.3)

The morphological alterations in consequence of the high temperature are well shown in the microphotographs of Plate 3.4. As regards the furrows, pictures 13 and 14 can be pointed out. The early non-saccate type of gymnosperm pollen which appeared secondarily is illustrated in Plate 3.4., fig. 49, 50.

The saccus degradation process (Text-fig. 3.1.) is similar to the previous species, *Pinus silvestris* L. The non-experimental and the heated pollen grains during one hour 2, 3, 4 and 5 hours are very similar. Most of the pollen grains are bisaccate very few pollen grains with one saccus in consequence of the degradation. At this species also as first important change at heating during 10 hours can be established. Important is the appearance of the saccus lost forms. The increase of the quantity of the non-saccate forms is near regular, except the experiment of 50 hours.

The results of the measurements are the following (Text-fig. 3.2.).

Corpus breath

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	40.0	47.5	55.0	15.0 μm
1h	35.0	40.0	45.0	10.0 μm
2h	32.5	37.5	45.0	12.5 μm
3h	32.5	37.5	42.5	10.0 μm
4h	30.0	35.0	40.0	10.0 μm
5h	32.5	35.0	40.0	7.5 μm
10h	27.5	37.5	50.0	22.5 μm
25h	25.0	27.5	40.0	15.0 μm
50h	25.0	27.5	32.5	7.5 μm
100h	17.5	27.5	32.5	15.0 μm
200h	20.0	22.5	25.0	5.0 μm
300h	15.0	22.5	25.0	10.0 μm

Three groups of graphs of the experimental material can be established. There is a remarkable difference between the graph of the non-experimental and those heated during one hour. The alterations until five hours of heating are more or less regular. The graph of the experiment during 10 hours is inside this group, but its character is completely different. The maximum is low, but its value is at the experiment of 3 hours. The heated pollen grains during 25, 50 and 100 hours have maxima at the same value, but the alteration is irregular. The variation-statistical graphs of experiments of 200 and 300 hours are near the same.

The dislocation of the non-experimental, and the experimental graph is exactly at 25 hours of heating.

The distances in μm between the maximum and minimum values vary from 22.5 μm until 5.0 μm. Extreme is at heating during 10 hours (22.5 μm) and at 200 hours (5.0 μm).

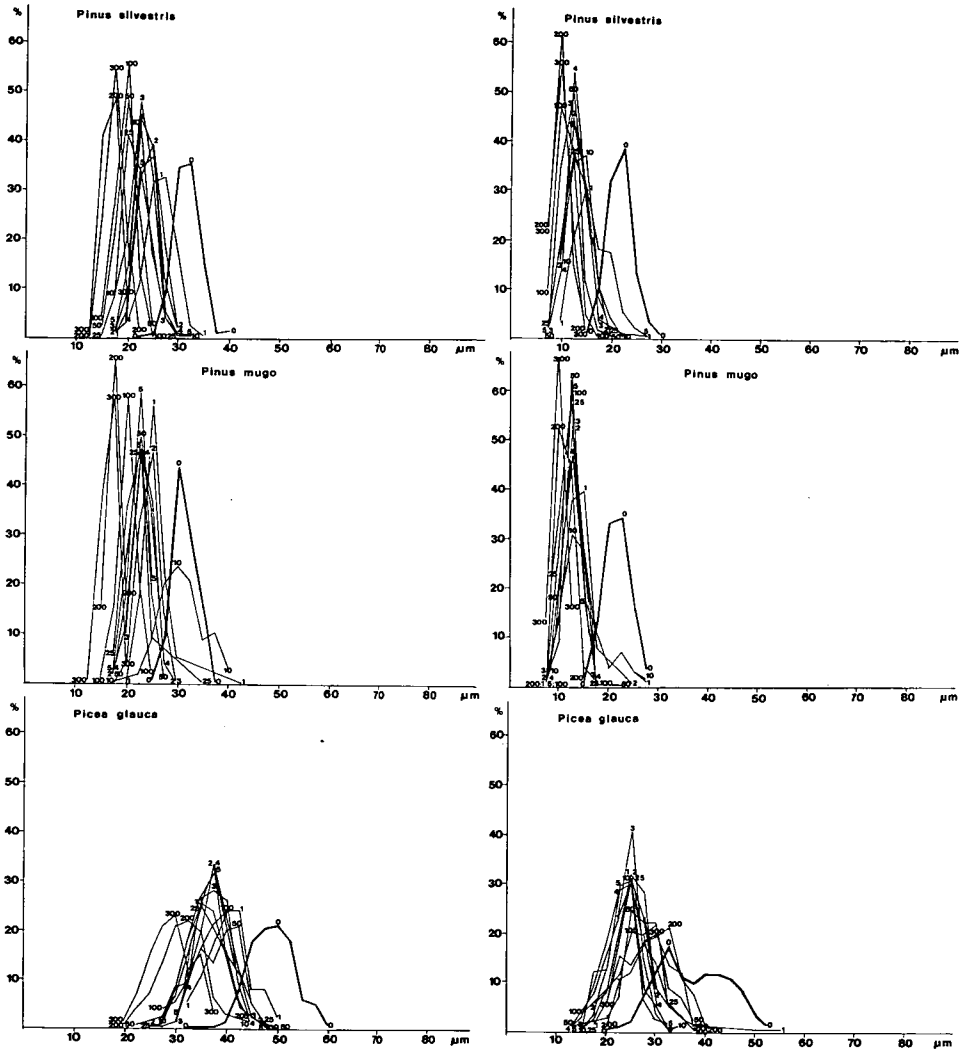
Corpus height

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	30.0	35.0	40.0	10.0 μm
1h	17.5	27.5	32.5	15.0 μm
2h	17.5	22.5	32.5	15.0 μm
3h	17.5	22.5	32.5	15.0 μm
4h	17.5	25.0	30.0	12.5 μm
5h	17.5	22.5	30.0	12.5 μm
10h	17.5	30.0	37.5	20.0 μm
25h	15.0	20.0	25.0	10.0 μm
50h	12.5	20.0	27.5	15.0 μm
100h	12.5	17.5	25.0	12.5 μm
200h	10.0	15.0	20.0	10.0 μm
300h	10.0	15.0	20.0	10.0 μm

The variation-statistical graphs are separated not so characteristically as at the corpus breath. The experiments of heating during 1—5 hours represent more or less one units, but the alterations are not regular. In this respect the heated pollen grains during 10 hours are also irregular. The values of heating at 25, 50 and 100 hours are very similar. The experiments during 200 and 300 hours are practically identical.

The dislocation of the non-experimental and experimental graphs is interesting. It is at about 20 hours. But the largest size of the experiments during 4 and 5 hours (30 μm) is the same at the smallest specimen of the non-experimental pollen grain.

The distances in μm between the maximum and minimum values vary from 20.0 μm until 10.0 μm. There is one extreme value, at 10 hours of heating.



Text-fig. 3.3.

Variation-statistical graphs of the alterations of the saccus width and height in consequence of high temperature. The numbers at the graphs indicate the length of times of heating at 200 °C.

Saccus width

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0^h	25.0	30.0	37.5	12.5 µm
1^h	20.0	25.0	42.5	22.5 µm

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
2h	17.5	25.0	30.0	12.5 μm
3h	20.0	22.5	30.0	10.0 μm
4h	17.5	22.5	27.5	10.0 μm
5h	17.5	22.5	25.0	7.5 μm
10h	17.5	30.0	40.0	22.5 μm
25h	17.5	22.5	35.0	17.5 μm
50h	17.5	22.5	27.5	10.0 μm
100h	15.0	20.0	25.0	10.0 μm
200h	15.0	17.5	20.0	5.0 μm
300h	12.5	17.5	20.0	7.5 μm

The variation-statistical graphs are nearly in one group. A particular exception is the experiment during 10 hours. Its maximum is at the value of the non-experimental. The maximum of experiments of 1—25 hours (except the above mentioned experiment during 10 hours) are nearly in one group. The maximum of the experiment during 100 hours is a little "isolated", but the variation-statistical graphs of the saccus width of the pollen grains heated during 200 and 300 hours are approximately identical.

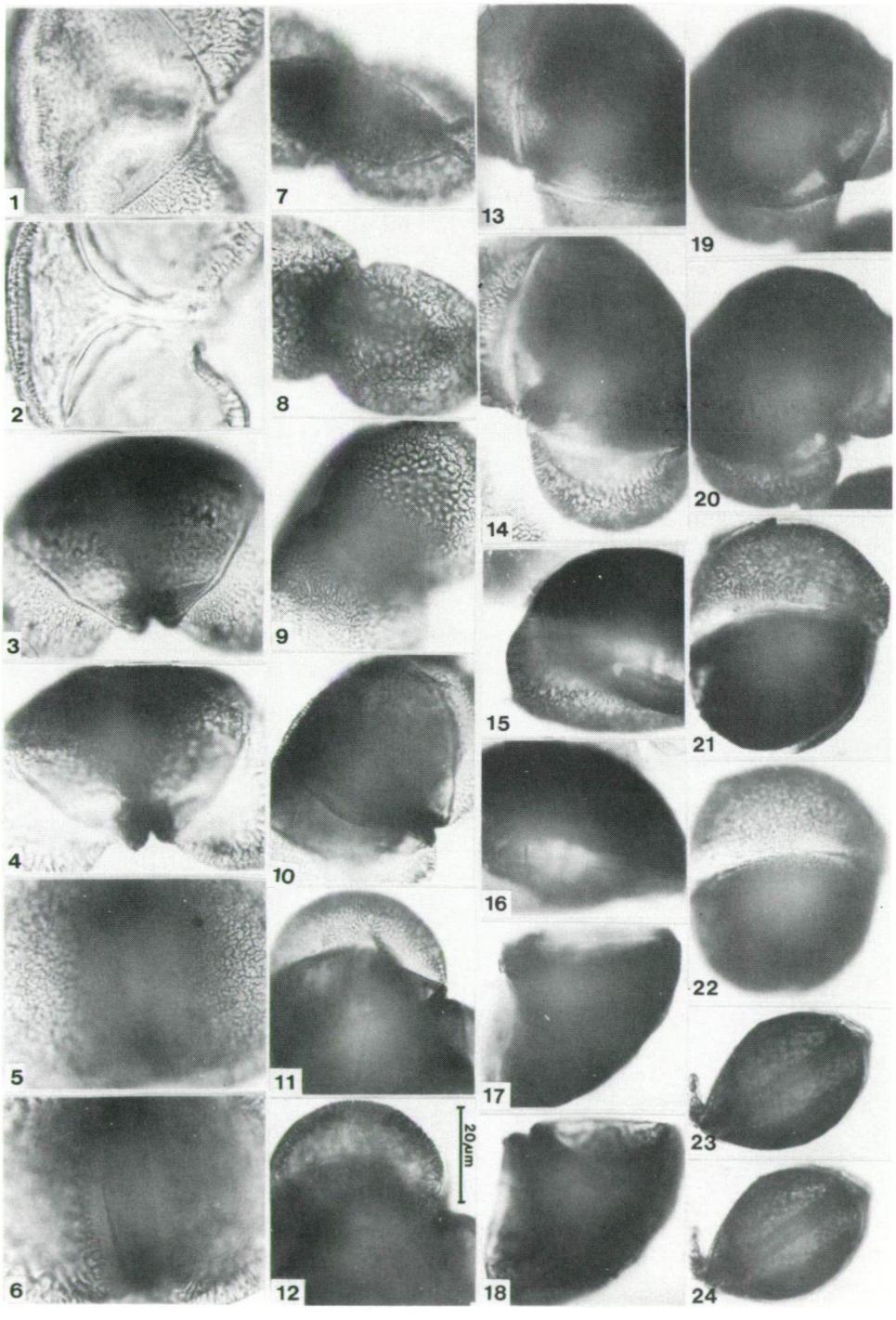
The dislocation of the non-experimental, and the experimental graphs are exactly at 25 hours of heating.

The distance in μm between the maximum and minimum values vary from 22.5 μm until 5.0 μm. Extreme values are at heating during one and 10 hours (22.5 μm) respectively at the experiment of 200 hours (5.0 μm).

Saccus height

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	15.0	22.5	27.5	12.5 μm
1h	7.5	15.0	27.5	20.0 μm
2h	7.5	12.5	17.5	10.0 μm
3h	7.5	12.5	17.5	10.0 μm
4h	7.5	12.5	17.5	10.0 μm
5h	7.5	12.5	15.0	7.5 μm
10h	7.5	12.5	27.5	20.0 μm
25h	10.0	12.5	17.5	7.5 μm
50h	10.0	12.5	22.5	12.5 μm
100h	7.5	12.5	17.5	10.0 μm
200h	7.5	10.0	15.0	7.5 μm
300h	7.5	10.0	12.5	5.0 μm

The variation-statistical graphs of the experimental pollen grains are in a relatively narrow group. Experiments during one hour 2, 3 and 5 hours have regularly altered graphs. Materials heated for 4, 10, 25, 100 and 50 hours are irregular. The isolation of the variation-statistical graph of the experiment during 10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hours is inside the above mentioned group. Worth of mentioning is that the maximum size of the non-experimental and heated during one hour and 10 hours are at the same value. The maxima of experiments during 200 and 300 hours are at the same value, but the per cents are quite different in contrast to the previous.

The dislocation of the non-experimental, and experimental material is interesting. Really it is at heating during 200 hours, but this value appears also at the experiment during 5 hours.

The distances in μm between the maximum and minimum values vary between 20.0 μm and 5.0 μm. As extreme value the experiment during one hour and 10 hours may be pointed out.

Picea glauca (MOENCH.) VOSS.
(Plate 3.5., figs. 1—24, plate 3.6., figs. 1—27, text-fig. 3.1., 3.2., and 3.3)

The morphological alterations, which appeared in consequence of the high temperature, are illustrated in two plates (Plate 3.5., and 3.6.). The apertural area is very characteristic, e.g.: Plate 3.5., fig. 3,4, plate 3.6., fig. 1,2, 11, 12, 22, 23. The degradation processes of the sacci are illustrated. The non-saccate forms may be triangular (Plate 3.6., fig. 11, 12), or nearly isodiametric (Plate 3.6., fig. 7,8). The "secondary monosulcate" form is not so characteristic as at the species of *Pinus*.

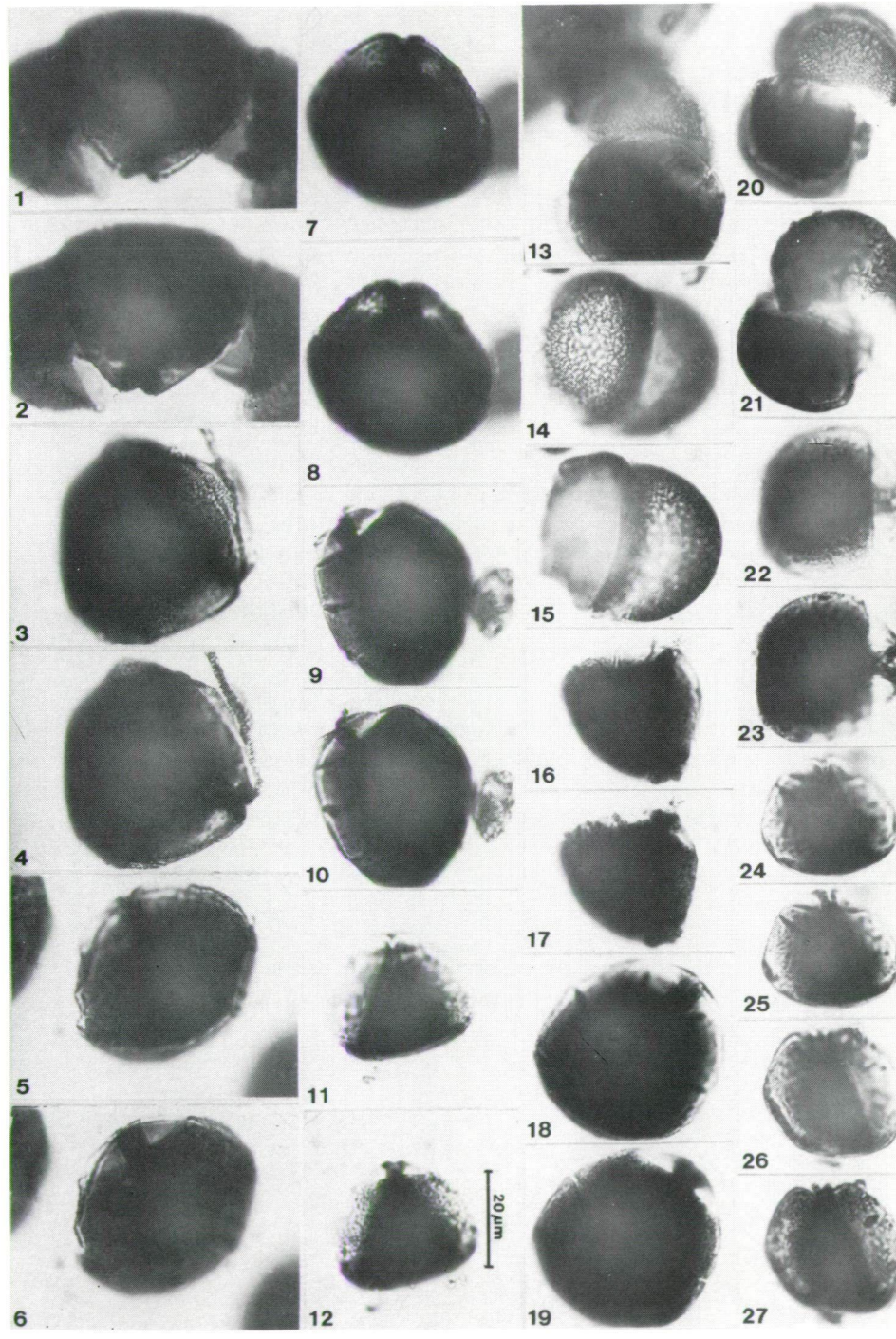
The quantitative data about the saccus degradation may be summarized in the following (Text-fig. 3.1.).

After one hour of heating, a remarkable decrease can be established at the bisaccate forms. But the heating during two hours does not resulted in important alterations in contrast to the experiment of one hour. The graphs of experiments during 3,4, and 5 hours represent another group. Important alterations are in contrast to the previous, namely the non-saccate forms appeared. The increasing of the per cents of the pollen grains without saccus is regular. Particularly peculiar is the variation-statistical graph of the experiment of 10 hours. Heating during 25, 100, 50, 200 and 300 hours resulted gradually saccus lost pollen grains. This alteration is nearly regular.

The results of the measurements can be summarized as follows.

◀ Plate 3.5.

1—24. *Picea glauca* (MOENCH.) VOSS., Recent.
1, 2. Pollen grains without staining or heating.
3, 4. Experiment No 351, length of time 1 hr.
5, 6. Experiment No 351, length of time 1 hr.
7, 8. Experiment No 352, length ot time 2 hrs.
9, 10. Experiment No 352, length of time 2 hrs.
11, 12. Experiment No 353, length of time 3 hrs.
13, 14. Experiment No 354, length of time 4 hrs.
15, 16. Experiment No 354, length of time 4 hrs.
17, 18. Experiment No 354, length of time 4 hrs.
19, 20. Experiment No 355, length of time 5 hrs.
21, 22. Experiment No 355, length of time 5 hrs.
23, 24. Experiment No 355, length of time 5 hrs.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◀ Plate 3.6.

1–27. *Picea glauca* (MOENCH.) VOSS., Recent.
1, 2. Experiment No 906, length of time 10 hrs.
3, 4. Experiment No 906, length of time 10 hrs.
5, 6. Experiment No 906, length of time 10 hrs.
7, 8. Experiment No 909, length ot time 25 hrs.
9, 10. Experiment No 909, length of time 25 hrs.
11, 12. Experiment No 909, length of time 25 hrs.
13. Experiment No 912, length of time 50 hrs.
14, 15. Experiment No 912, length of time 50 hrs.
16, 17. Experiment No 912, length of time 50 hrs.
18, 19. Experiment No 758, length of time 100 hrs.
20, 21. Experiment No 758, length of time 100 hrs.
22, 23. Experiment No 915, length of time 200 hrs.
24, 25. Experiment No 918, length of time 300 hrs.
26, 27. Experiment No 918, length of time 300 hrs.

Corpus breath

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	52.5	62.5/72.5	82.5	30.0 µm
1h	52.5	65.0	75.0	22.5 µm
2h	50.0	62.5	72.5	22.5 µm
3h	50.0	60.0	70.0	20.0 µm
4h	52.5	60.0	70.0	17.5 µm
5h	50.0	57.5	67.5	17.5 µm
10h	45.0	55.0	75.0	30.0 µm
25h	40.0	50.0	67.5	27.5 µm
50h	27.5	45.0	57.5	30.0 µm
100h	32.5	47.5	52.5	20.0 µm
200h	30.0	37.5	50.0	20.0 µm
300h	30.0	37.5	52.5	22.5 µm

The variation-statistical graphs of the heated pollen grains well represent the decrease in size of the corpus. The alterations are regular until 25 hours of heating. The graph of the experiment during 100 hours is irregular, with two maxima. The highest points of the experiments during 200 and 300 hours are at the same value. But the two graphs are quite different.

The dislocation of the non-experimental, and experimental graphs is at heating during 100 hours. This value appears newly at the experiment during 300 hours.

The distances between the maximum and minimum values vary from 30.0—17.5 µm.

Corpus height

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	42.5	50.0	62.5	20.0 μm
1h	30.0	40.0	55.0	25.0 μm
2h	30.0	35.0/40.0	52.5	22.5 μm
3h	27.5	40.0	50.0	22.5 μm
4h	27.5	37.5	50.0	22.5 μm
5h	30.0	40.0	50.0	20.0 μm
10h	25.0	37.5	50.0	25.0 μm
25h	25.0	37.5	47.5	22.5 μm
50h	20.0	37.5	45.0	25.0 μm
100h	17.5	30.0	45.0	27.5 μm
200h	20.0	32.5	40.0	20.0 μm
300h	20.0	30.0	37.5	17.5 μm

The variation-statistical graphs of the experimental pollen grains represent two groups. These are not characteristically separated. The sizes of the pollen grains heated during one hour, 2, 3, 4, 5, 10, 25 and 50 hours are similar to each other. In this case, the variation-statistical graphs of the pollen grains heated during 100, 200 and 300 hours are also similar.

The dislocation of the non-experimental, and the experimental graphs is between heating during 100 and 200 hours.

The distances between the maximum and minimum values vary from 27.5 μm, until 17.5 μm, mostly 20.0 μm and 22.5 μm. As extreme value the heating during 100 hours may be pointed out (27.5 μm).

Saccus width

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	32.5	50.0	60.0	27.5 μm
1h	32.5	40.0/42.5	50.0	17.5 μm
2h	25.0	37.5	47.5	22.5 μm
3h	30.0	37.5	45.0	15.0 μm
4h	32.5	37.5	45.0	12.5 μm
5h	30.0	37.5	47.5	17.5 μm
10h	27.5	35.0	45.0	17.5 μm
25h	25.0	35.0	47.5	22.5 μm
50h	22.5	42.5	50.0	27.5 μm
100h	27.5	40.0	47.5	20.0 μm
200h	20.0	32.5	45.0	25.0 μm
300h	20.0	30.0	37.5	17.5 μm

The variation-statistical graphs are very interesting. Heating during one hour, 50 and 100 hours represents the first group. The maximum values of the experiments

during 2, 4 and 5 hours are practically the same, but the graph of 3 hours is also in this region. The maxima of the variation-statistical graph of heating during 10 and 25 hours are opposite to those of the one and 100 hours. Heating during 200 and 300 hours resulted in similar, but not identical, graphs.

The dislocation of the non-experimental, and the experimental graphs has not happened after 300 hours of heating.

The distances between maximum and minimum values vary from 27.5 μm until 12.5 μm. This latter mentioned, extreme value is at 4 hours of heating.

Saccus height

Length of time of heating	Smallest	Sizes dominant in quantity	Largest	Distance between smallest and largest specimens
0h	20.0	32.5	52.5	32.5 μm
1h	17.5	25.0	55.0	37.5 μm
2h	17.5	25.0	30.0	12.5 μm
3h	15.0	25.0	32.5	17.5 μm
4h	12.5	25.0	30.0	17.5 μm
5h	12.5	25.0	32.5	20.0 μm
10h	15.0	25.0	35.0	20.0 μm
25h	15.0	25.0	32.5	17.5 μm
50h	12.5	25.0	37.5	25.0 μm
100h	15.0	30.0	37.5	22.5 μm
200h	20.0	32.5	40.0	20.0 μm
300h	20.0	30.0	37.5	17.5 μm

The variation-statistical graphs are in every respect irregular. The experiments of one hour 2, 3, 5, 10 and 25 hours are in the same group. The graph of heating during 3 hours is different.

The maximum of the graph of 200 hours is at the same value, as the non-experimental. The maximum of heating during 300 hours is also not so far from the above mentioned ones.

Similarly to the saccus width, the dislocation of the non-experimental, and the experimental graphs has not happened.

The distance between the maximum and minimum values vary from 37.5 μm until 12.5 μm.

Discussion and Conclusions

The degradation process of the saccus is interesting and irregular, as it was described previously. There are some peculiar lengths of time of heating, as it was described previously. Probably as an important fact, the appearance of the saccus lost forms can be pointed out. These as extremely altered secondary forms are very important in the determination of the fossil forms, particularly in the more or less metamorphic layers. This problem was discussed in our several earlier papers, new data can be summarized as follows.

i. The extremely altered bisaccate pollen grains may be similar to altered algal cysts.
ii. These above mentioned forms may also be similar to the thermal altered forms of the genus *Equisetum.*
iii. It is interesting that the so-called monosulcate forms similar to the early Mesozoic gymnosperms (cf. *Bennettitaceaeacuminella* MALYAVKINA 1953, *Gynkgaletes* LYUBER 1955) appear, probably indicating the ancestors of the earliest — Paleozoic — stage of evolution. Worth of mentioning is that these forms are mostly characteristic to the pollen grains of the genus *Pinus,* but occurred sporadically among the altered pollen grains of *Picea glauca* too.
iv. In general the apertural area developed more characteristically after several heating. It may be presumed that some altered forms can be determined as extremely early angiosperm pollen grain, in the fossil palynological associations.
v. The appearance of the triangular forms firstly at the pollen grains of *Picea glauca* indicates also angiosperm-like forms.

Concerning the quantitative data the following can be pointed out:

i. There are also irregularities in the dimensional alterations of the corpus and the saccus.
ii. The variation-statistical graphs may be arranged into groups, these also can be regular or irregular.
iii. The dislocation point (length of time of heating) of the variation-statistical graphs are as follows.

	Corpus		Saccus	
	breath	height	width	height
Pinus silvestris	25^h	25^h	200^h	200^h
Pinus mugo	25^h	about 20^h	100^h	200^h
Picea glauca	$100-(300)^h$	$100-200^h$	0^h	0^h

The results of the saccus at *Picea glauca* are unusual and interesting.

Acknowledgements

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4. TRANSMISSION ELECTRON MICROSCOPY OF PARTIALLY DISSOLVED EXINES OF DIFFERENT BISACCATE GYMNOSPERM POLLEN GRAINS

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Abstract

In the present paper our TEM observations are published on the bisaccate gymnosperm pollen grains treated previously with different organic solvents. The diethylether is a suitable solvent for studying the biopolymer structure of the inner exine layers: foot layer, endexine and intine. The glycogen molecular structure may be discovered with tetrahydrofuran. N-pentane, and particularly pyrrolidine is the best to dissolve the quasi-crystalloid skeleton of the exine, probably of the plant cell wall. This is a tool to investigate the stabilizing biopolymer system. The detailed study of the stabilizing molecular structures will be the subject of further investigations.

Key words: Palynology, bisaccate gymnosperm, biopolymer structure.

Introduction

The discovery of the quasi-crystalloid biopolymer skeleton in the plant cell wall, first described in the exine of the pollen grains of *Pinus griffithii* McCLELL in 1988, raised several problems. The most important are as follows.

1. Methodical investigations in two different, opposite ways;

1.1. The TEM study of the quasi-crystalloid skeleton after dissolving or oxidizing the so-called stabilizing molecular structures. In these experiments we have the opportunity to demonstrate the different levels of organization.

1.2. To dissolve the quasi-crystalloid skeleton, to get more information about the stabilizing biopolymer structures. By the modified MARKHAM rotation method, the "negative PENROSE modell" was planned as another way for the verification of the quasi-crystalloid system of the plant cell wall.

2. Comparisons between recent and fossil data, inside them the different kinds of plant cell wall, not only the "sporopollenin type" biopolymer structures.

3. To pay particular attention to the outer and inner surfaces and to the previously established characteristic features, such as the molecular sieve character (ROWLEY, 1973), and the electrostatic charge of the surface (ROWLEY, 1971).

4. As it was emphasized in our previous paper (KEDVES, 1991) the three dimensional modelling is a necessity and now it seems promising to project

several parallel studies, because these results may have favourable effect on each other.

During one of our new research program the basic concept is to dissolve the quasi-crystalloid biopolymer skeleton. Concerning this subject we have few information. Some data were published in our preliminary report (KEDVES et al., 1991), and in another paper (KEDVES and ROJIK, 1991) on the sclereids of *Armeniaca vulgaris* GAERTN.

Regarding the details the following may be pointed out.

1. Investigating the effect of one solvent on different taxa.
2. The effect of the different solvents on one species and/or sample.
3. Varia.

Material and Methods

The following species were the subject of our investigations:

1. *Pinus mugo* TURRA
   Coll.: Dr. K. MARGÓCZI, Botanical Garden of the J. A. University, 18. 5. 1988.
2. *Pinus silvestris* L.
   Coll.: Dr. K. MARGÓCZI, Botanical Garden of the J. A. University, 18. 5. 1988.
3. *Pinus griffithii* McCLELL
   Coll.: Dr. K. MARGÓCZI, Botanical Garden of the J. A. University, 18. 5. 1988.
4. *Picea glauca* (MOENCH.) VOSS.
   Coll.: Dr. K. MARGÓCZI, Botanical Garden of the J. A. University, 18. 5. 1988.

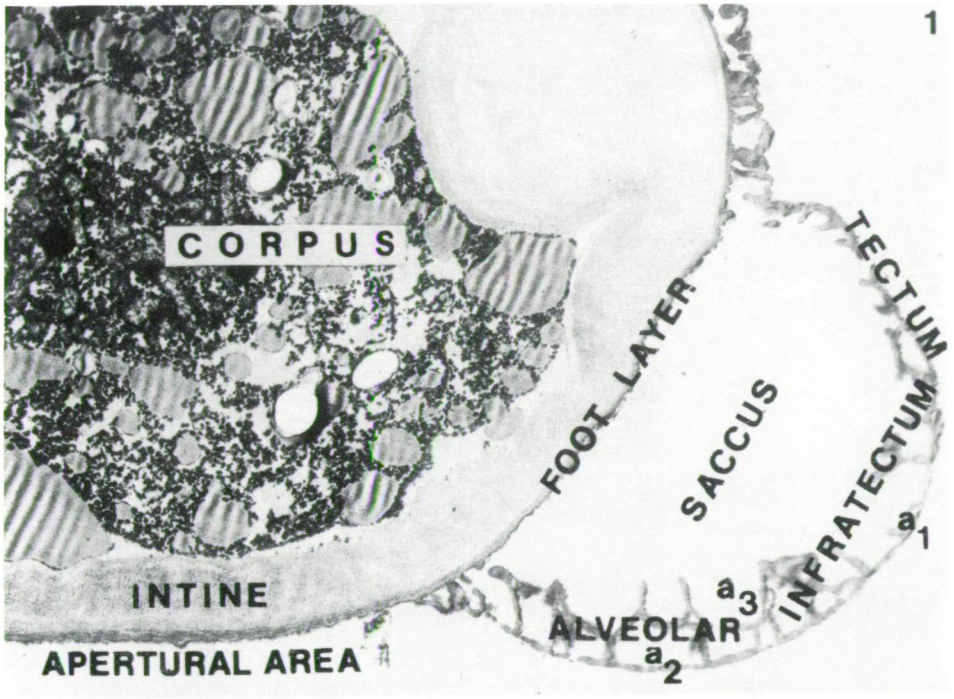
The experiments were made as follows.

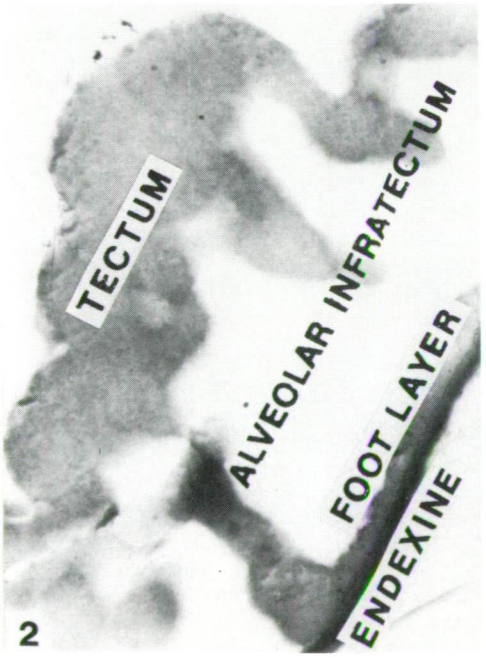
	tetrahydrofuran	n-pentane	pyrrolidine	diethylether
Pinus mugo	634	656	670	682
Pinus silvestris		657		692
Pinus griffithii			669	681
Picea glauca			671	680

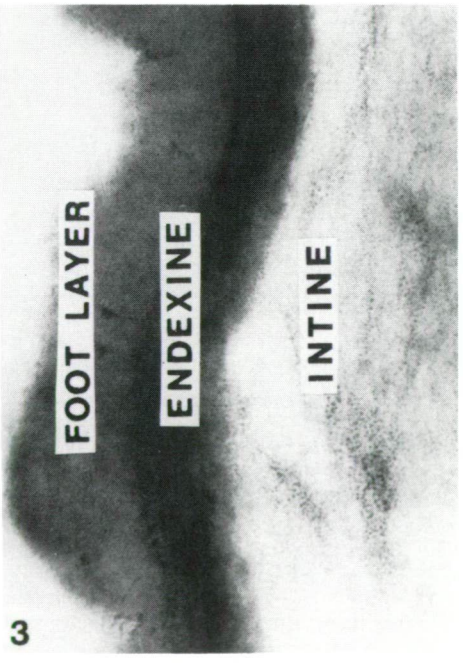
20 mg air dried pollen grains+5 ml solvent. The experiment started on 10. 5. 1989 at 16^h, and finished on 24. 5. 1989 at 10^h. Temperature from 10. 5. 1989, 16^h up to 12. 5. 1989 $8^{30'}$ the so-called room temperature after in a refrigerator on +5—+6 °C. The residue material was washed in aethyl alcohol, and fixed in 1% OsO_4 aq. dil., embedded in Araldite. The ultrathin sections were made in the EM Laboratory of the Biological Research Center of the Hungarian Academy of Sciences on a Porter Blum ultramicrotome. The TEM pictures were taken on a TEM instrument of Opton EM—902 (resolution 2—3.5Å).

Submicroscopic morphological nomenclature of the bisaccate pollen grains

Several kinds of nomenclature are known in this subject. The most important establishments obtained firstly on the basis of TEM data are summarized in the following. Concerning their general morphology (POCKNALL, 1981, and HANSEN and ENGSTROM, 1985) the pollen grain consists of corpus (sensu strictu pollen grain) and of saccus (Plate 4.1., fig. 1). The apertural area is on the distal part, this is a furrow. On the border of the saccus/corpus there is the furrow rim, on the proximal part the cappa (cappus) and the marginal ridge are found. The infratectal layer including the corpus and the sacci is characteristically alveolar (cf. M. VAN CAMPO, 1973). At this place it is necessary to point out that the saccus is not reticulate as it was written in several papers. The former light microscopic description is misleading because the optical section of the alveoli forms seemingly a reticulum. The alveolar structure is essentially a system of lamellar infratectal elements. The orientation of these lamellae may be regular or irregular. Concerning the saccus, following M. VAN CAMPO (1973, p. 98): "On peut dans les Sapins distinguer 3 couches d'alvéoles, les alvéoles a_1 petits fermés directement sous le tectum, les alvéoles


1
CORPUS
FOOT LAYER
SACCUS
TECTUM
INFRATECTUM
a_1
a_3
INTINE
ALVEOLAR
a_2
APERTURAL AREA


TECTUM
ALVEOLAR INFRATECTUM
FOOT LAYER
ENDEXINE
2


FOOT LAYER
ENDEXINE
INTINE
3

moyens a_2 et les grands alvéoles internes, ouverts a_3." KURMANN (1989) established as follows, p. 2489: "...there are differences in the pollen wall formation between gymnosperms and angiosperms especially in the timing of the wall deposition." P. 2502: "In angiosperms, the ektexine is formed during the tetrad phase and the endexine, if present, in the free spore phase. In gymnosperms, however, both the ektexine and endexine are deposited during the tetrad phase." Following VASIL (1978), p. 118: "...the intine is formed immediately outside the plasmolemma, GOLGI-derived vesicles are again involved in the deposition of the intine, which is a homogeneous layer devoid of any lamellations or fibrillar substructure." MARTENS and WATERKEYN (1961), p. 1390: "...intine — c'est-à-dire la vrai membrane cellulaire — est encore très mal connue." P. 1393: "Nous admettons aussi avec Mme VAN CAMPO (1950), que l'intine de *Picea* est mince et simple, celle de *Pinus* épaisse et complexe,..."

We hope that these above mentioned basic establishments are enough for the interpretation and discussion of our experimental studies.

◀ Plate 4. 1.

1—3. *Pinus mugo* TURRA.
Ultrastructure of the pollen grains after partial degradation with diethylether. Experiment No: 682.

1. General survey picture of the ultrastructure of the pollen grain. Negative no: 808, 2.500x.
2. Fine structure details of the exine of the corpus. Negative no: 816, 25.000x.
3. Ultrastructure of the inner layers of the exine. Negative no: 817, 100.000x.

Results

1. INVESTIGATION OF THE EFFECT OF ONE SOLVENT (DIETHYLETHER) TO DIFFERENT TAXA

Pinus mugo TURRA
Experiment No: 682
(Plate 4.1., figs. 1—3, plate 4.2., figs. 1—5)

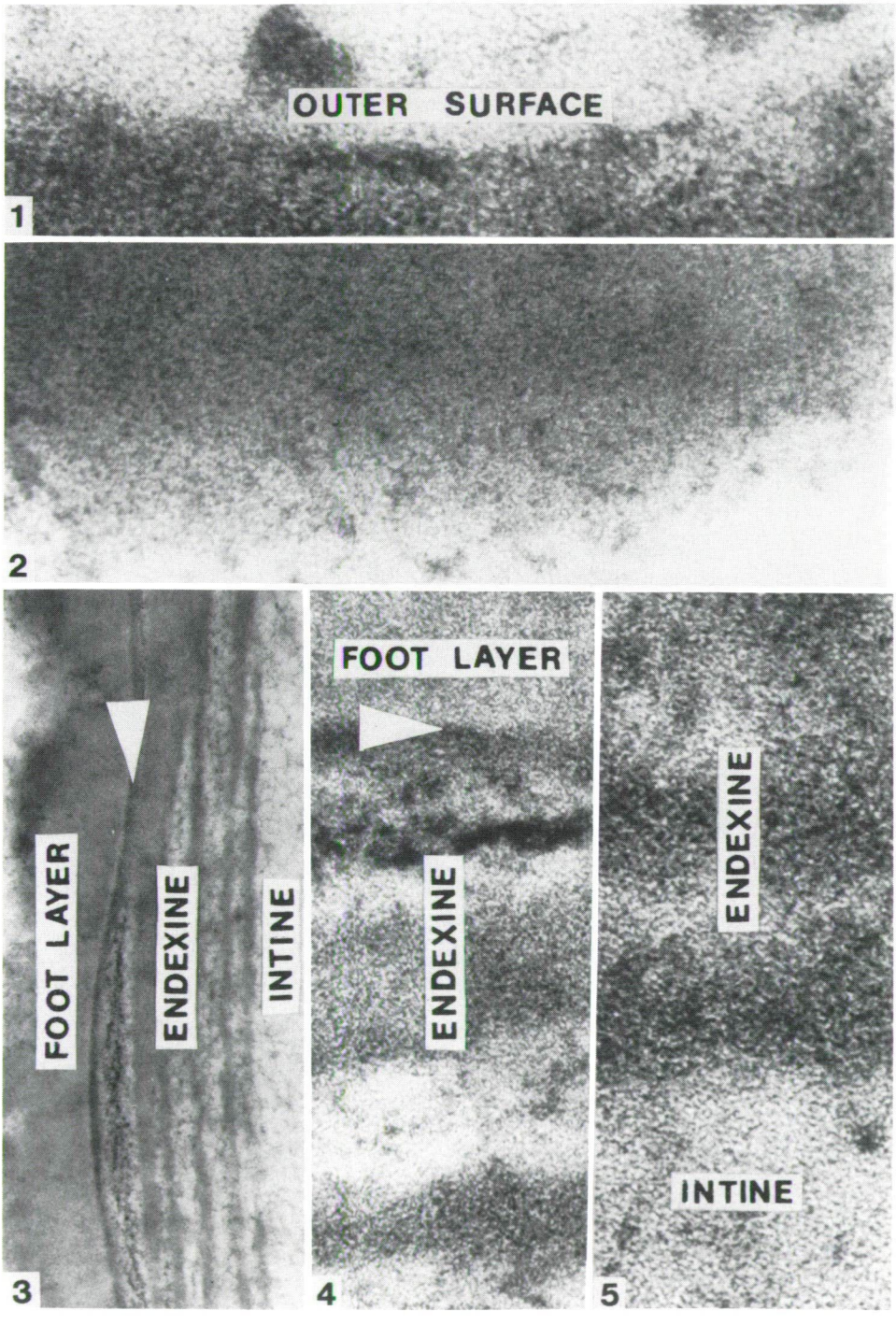
In low magnification (so-called general survey picture) (Plate 4.1., fig. 1) the cytological characteristic features of the pollen grain ultrastructure of the proximal and distal surfaces, and those of the saccus are well illustrated. The three kinds of the saccus alveoli, mentioned previously in the paper of M. VAN CAMPO (1973) are also clearly shown. The intine is extremely thick. In the apertural area the ectexine consists of the foot layer only.

Corpus (Plate 4.1., fig. 2, 3)

Fig. 2, illustrates the exine stratification. The foot layer is relatively thin, and the endexine distincts only by its stronger electron density. The finer stratification of the inner layers of the corpus is as follows (Plate 4.1., fig. 3). The endexine has a very strong electron density but there are less characteristic lamellations, too. The endexine/intine border is a lighter part. In the intine fine lamellations have been observed. As regards the biopolymer organization of the exine layers of the corpus this experiment has not yielded sufficient data.

Saccus (Plate 4.2., figs. 1—5)

Tectum (Plate 4.2., fig. 1)


OUTER SURFACE
1
2
FOOT LAYER
ENDEXINE
INTINE
3
FOOT LAYER
ENDEXINE
4
ENDEXINE
INTINE
5

On the surface of the tectum this experiment demonstrated characteristic surface — protective — layer of molecules composed of globular units, connected with tiny arms. The diameter of the granular elements range from 3—7 Å, mostly 3—5 Å. The granular biopolymer units of the inner part of the tectum are a little larger; 3—13 Å (mostly 6—8 Å). The size of the granular elements of the inner surface of the tectum is similar to the previously mentioned; 4—11 Å, mostly 4—8 Å, in this way, it differs from the biopolymer organization of the surface. These filaments can correspond to the glycogen molecular structure (network of chains, cf. DARNELL et al., 1986), but are of different degrees of degradation.

Infratectal layer (Plate 4.2., fig. 2)

The globular biopolymer units are of 4—10 Å in diameter. These elements have often fibrillar or irregular network-like arrangement.

Foot layer (Plate 4.2., fig. 3)

The surface of this layer is also covered with granular biopolymer units of 4—10 Å in diameter (mostly 6—8 Å). The border with the lamellar endexine is characteristic. A narrow bright and a dark layer separate well these two parts, marked with arrows (Plate 4.2., fig. 3, 4).

Endexine (Plate 4.2., figs. 3—5)

This layer is characteristically lamellar, composed of compact and loose layers. The compact lamellae are similar to the foot layer or in general to the endexine, the loose part to the intine. The globular biopolymer units of the compact part of the endexine range from 3 to 10 Å (mostly 6—8 Å), those of the loose part; 4—10 Å (mostly 4—8 Å).

Intine (Plate 4.2., figs. 3—5)

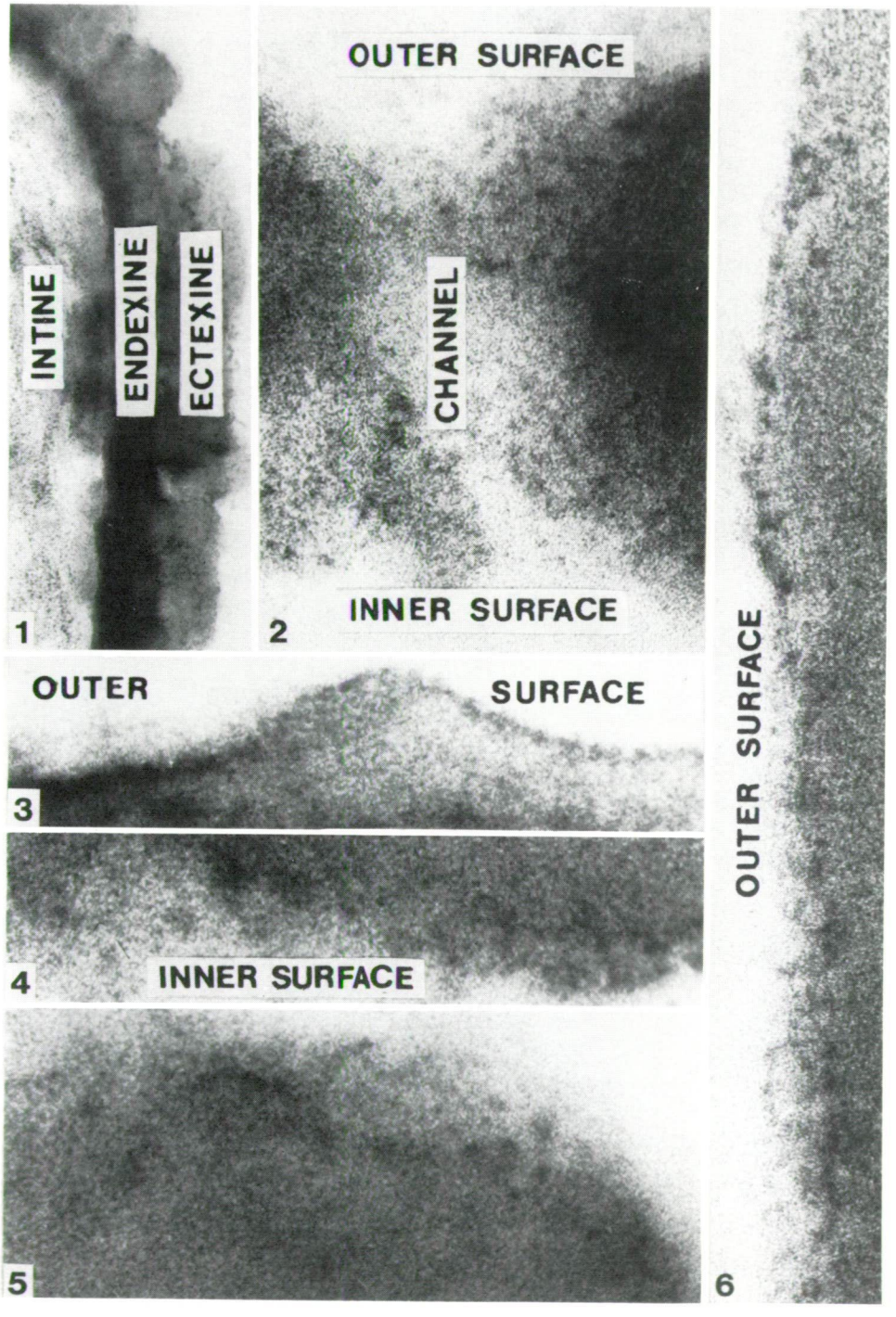
The globular elements of this layer are relatively small, 2—7 Å (mostly 3—4 Å), and are sometimes of linear or network-like arrangement.

Pinus silvestris L.
Experiment No: 693
(Plate 4.3., figs. 1—6)

◀ Plate 4.2.

1—5. *Pinus mugo* TURRA.
Ultrastructure of the saccus of the pollen grains after partial degradation with diethylether. Experiment No: 682.

1. Biopolymer organization of the tectum of the saccus. Negative no: 810, Magnification 1 million.
2. Biopolymer structure of the infratectum of the saccus. Negative no: 811, 500.000x.
3. Ultrastructure of the inner layers of the saccus. Negative no: 813, 100.000x.
4. The border of the ectexine and endexine marked with an arrow. Negative no: 814, 500.000x.
5. The border of the endexine and the intine of the saccus. Negative no: 815, Magnification 1 million.


INTINE
ENDEXINE
ECTEXINE
1
OUTER SURFACE
CHANNEL
INNER SURFACE
2
OUTER
SURFACE
3
INNER SURFACE
4
5
OUTER SURFACE
6

Corpus (Plate 4.3., fig. 1)

The TEM pictures are very similar to those of the previous experiment (cf. Plate 4.1., fig. 3), this can be consequence of the identic solvent.

Ectexine (Plate 4.3., fig. 1)

The three layers of the ectexine are not so characteristic. Small globular elements with relatively strong electron density are observed on the surface.

Endexine (Plate 4.3., fig. 1)

This layer is well separated from the ectexine by its strong electron density.

Intine (Plate 4.3., fig. 1)

Beneath the endexine a finely lamellar intine is found.

Saccus (Plate 4.3., figs. 2—6)

In this experiment the biopolymer structure of the pollen wall was particularly investigated.

Tectum (Plate 4.3., figs. 2—4, 6)

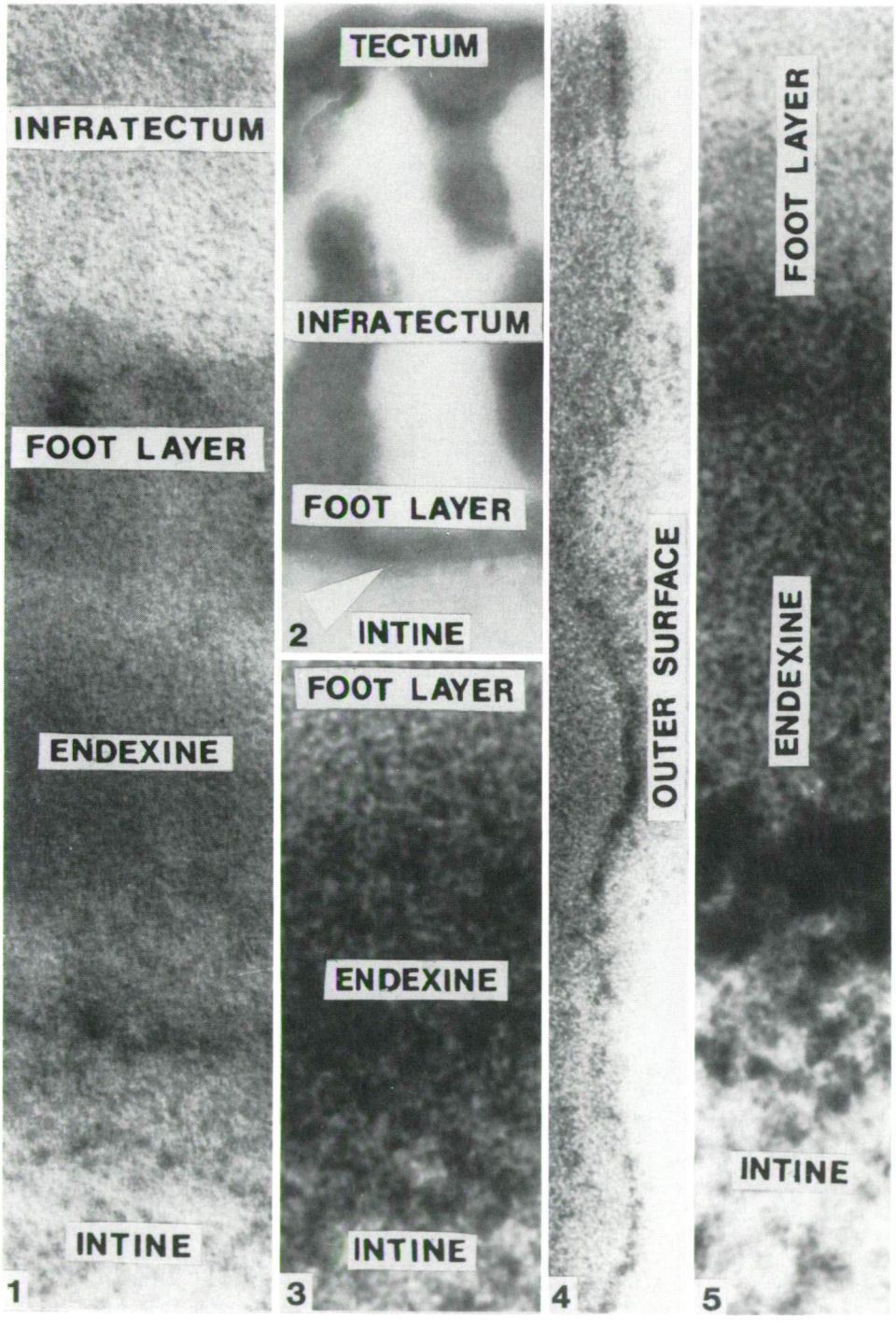
Fig. 2 on Plate 4.3. represents well the biopolymer structure of the tectum channel. The globular biopolymer units of the surface and the inner part of the tectum were investigated in the pictures of five TEM negatives. The summarized results of their size distribution are presented in the following chart. The first number represents the number of the biopolymer units of a given diameter of the surface, the second ones that of the inner part of the tectum.

Diameters in Å	Numbers of the negatives				
	818	824	825	826	827
3	3;0	9;0	1;0	2;0	2;0
4	6;1	4;5	5;3	5;1	6;0
5	5;1	2;2	5;2	4;2	0;3
6	4;7	9;5	11;6	6;6	8;5
7	1;0	3;1	6;1	1;0	6;1
8	4;5	9;8	7;4	4;7	11;9
9	0;1	4;0	0;2	2;0	1;1
10	1;5	5;9	5;5	1;10	5;7
11	0;1	0;2	0;4	0;5	0;7

◀ Plate 4.3.

1—6. *Pinus silvestris* L.
TEM pictures of the pollen grains after partial degradation with diethylether. Experiment No: 693.

1. The wall ultrastructure of the corpus. Negative no: 822, 100.000x.
2. The tectum of the saccus. Negative no: 818, 500.000x.
3, 4. The surfaces of the tectum of the saccus. Negative no: 825, 500.000x.
5. Biopolymer organization of the infratectum of the saccus. Negative no: 820, 500.000x.
6. Different kinds of preservation of the biopolymer structure of the outer surface of the tectum of the saccus. Negative no: 827, 500.000x.


INFRATECTUM
FOOT LAYER
ENDEXINE
INTINE
1
TECTUM
INFRATECTUM
FOOT LAYER
2
INTINE
FOOT LAYER
ENDEXINE
INTINE
3
OUTER SURFACE
4
FOOT LAYER
ENDEXINE
INTINE
5

It is shown that the globular units of the surface range from 3—10 Å (mostly 4—8 Å). Those of the inner part of the tectum: 4—11 Å, mostly 4—10 Å.

The protective biopolymer layer of the outer surface is well illustrated on fig. 3 and 6 of the Plate 4.3. Molecular disintegration was observed on the outer, inner and channel surfaces (Plate 4.3, fig. 2) and on the inner surface of the tectum (Plate 4.3., fig. 3).

Infratectum (Plate 4.3., fig. 3)

The disintegration of the biopolymer system was established on the basis of the TEM pictures.

The further layers of the exine were not investigated at this experiment with the TEM method.

Pinus griffithii McCLELL
Experiment No: 681
(Plate 4.4., figs. 1—5)

This species is extremely important in the knowledge of the biopolymer organization of the pollen exine.

Corpus (Plate 4.4., figs. 1—3)

The stratification of the exine is illustrated in the fig. 2. The foot layer is relatively thin. Beneath the foot layer there is a very thin endexine marked with an arrow. It is noteworthy, that after experiment, the intine is not lamellar. Concerning the biopolymer structure of the infratectum, foot layer, endexine and intine (Plate 4.4., fig. 1) the following can be pointed out. The delimitation of the infratectum and the foot layer is characteristic. The molecular structure which is more or less characteristic of the different inner layers, are illustrated in picture 3, Plate 4.4. The diameter distribution of the globular biopolymer structures of the different parts of the foot layer is summarized as follows. (The first number indicates the number of biopolymers of the outer surface, the second and third ones those of the inner part, and inner surface respectively.)

◀ Plate 4.4.

1—5. *Pinus griffithii* McCLELL.
TEM pictures of the pollen grains after partial degradation with diethylether. Experiment No: 681.

1. The biopolymer organization of the inner layers of the corpus. Negative no: 401, 500.000x.
2. General survey picture of the wall of the corpus after the diethylether treatment. The endexine is marked with an arrow. Negative no: 400, 50.000x.
3. Biopolymer organization of the inner layers of the wall of the corpus. Negative no: 429, Magnification 1 million.
4. Biopolymer structure of the surface of the tectum of the saccus. Negative no: 424, 500.000x.
5. Biopolymer organization of the inner layers of the saccus. Negative no: 432, Magnification 1 million.

Diameters in Å	Numbers of the negatives					
	401	402	403	428	429	432
2	5;6;1	1;0;19	5;9;0	1;0;4	0;0;2	0;16;0
3	4;2;0	4;0;0	2;0;0	4;3;1	0;1;14	0;1;14
4	1;0;5	9;0;13	2;4;0	1;2;3	0;2;10	0;7;5
5	10;4;2	0;0;1	0;0;1	4;2;7	0;5;12	0;5;10
6	5;6;6	7;0;11	5;15;4	14;12;18	0;7;4	0;6;12
7	2;0;1	0;0;0	1;2;0	0;0;10	0;2;5	0;1;1
8	4;7;10	4;0;13	2;10;9	6;6;12	0;6;6	0;1;14
9	0;0;0	0;0;1	0;0;0	0;0;2	0;2;3	0;0;1
10	0;4;5	0;0;2	0;0;9	10;4;7	0;5;2	0;0;5
11	0;0;3	0;0;0	0;0;1	0;0;1	0;0;0	0;0;0

The measured values are quite different, but essentially it can be concluded that the globular biopolymer units of the surface are a little larger than those of the outer surface and/or of the inner part of the foot layer. As regards the intine of the corpus, the measured values are as follows.

Diameters in Å	
2	0
3	0
4	10
5	7
6	30
7	12
8	37
9	4
10	16

In this way it may be concluded, that these granular elements are significantly larger than those of the inner parts of the ectexine and the intine.

Saccus (Plate 4.4., fig. 4, 5)

Tectum (Plate 4.4., fig. 4)

On the outer surface of the tectum, the protective molecular layer is well shown, it is composed of globular units of 2—9 Å (mostly 4—6 Å) in diameter. It is also well shown in picture 4 of Plate 4.4., that one part of this layer is damaged, and the destruction of the biopolymer system of the tectum has already started.

Infratectum (not illustrated)

The globular biopolymer units of this layer are of 2—10 Å (mostly 6 Å) in diameter.

Endexine (Plate 4.4., fig. 5)

This layer distincts well from the foot layer by its stronger electron density.

Intine (Plate 4.4., fig. 5)

The globular units of the intine in this picture are characteristic and separate quite well from the endexine.

Picea glauca (MOENCH.) VOSS.
Experiment No: 680
(Plate 4.5., figs. 1—4)

In case of this species the corpus and the corpus/saccus border were investigated in detail. The general survey pictures illustrate well the characteristic ultrastructural features of the pollen grain. Fig. 1 in the Plate 4.5., illustrates well the uneven outer surface of the tectum, the characteristic alveolar infratectal layer, and the relatively thin foot layer. At the bordering part of the corpus/saccus, between the lamellar endexine and the foot layer there is an interbedded layer with finely lamellar substance. The thickness of this layer is not uniform, this is particularly well shown in picture 2, of Plate 4.5. The endexine is lamellar. The endexine/intine border is not so clear. Regarding the details, the following can be pointed out.

Tectum (not illustrated in larger magnification)
On the surface of the tectum, the protective molecular film is well shown. The damage of this layer was also observed with the degradation of the biopolymer system of the tectum. The diameter of the globular biopolymer units is 3—10 Å, mostly 4—8 Å. The inner surface of the tectum including its biopolymer structure is similar to the outer one.

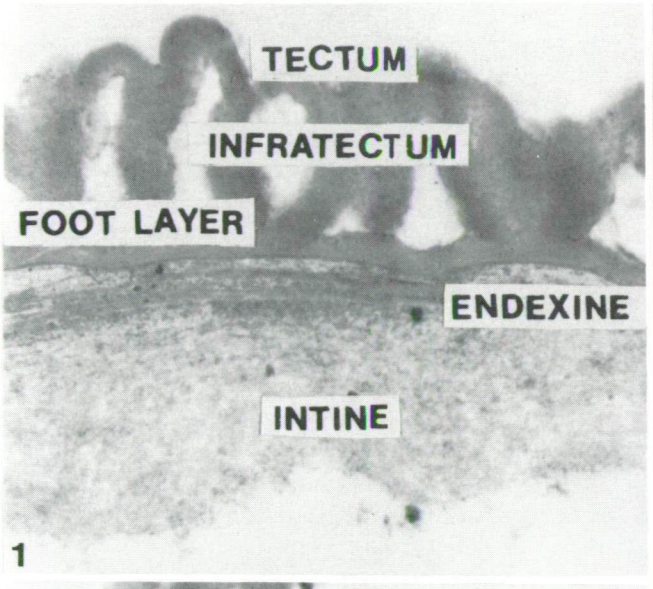
Infratectum (not illustrated in high magnification)
The observed globular biopolymer units are of 2—10 Å (mostly of 2—4 Å) in diameter, a little smaller than those of the tectum.

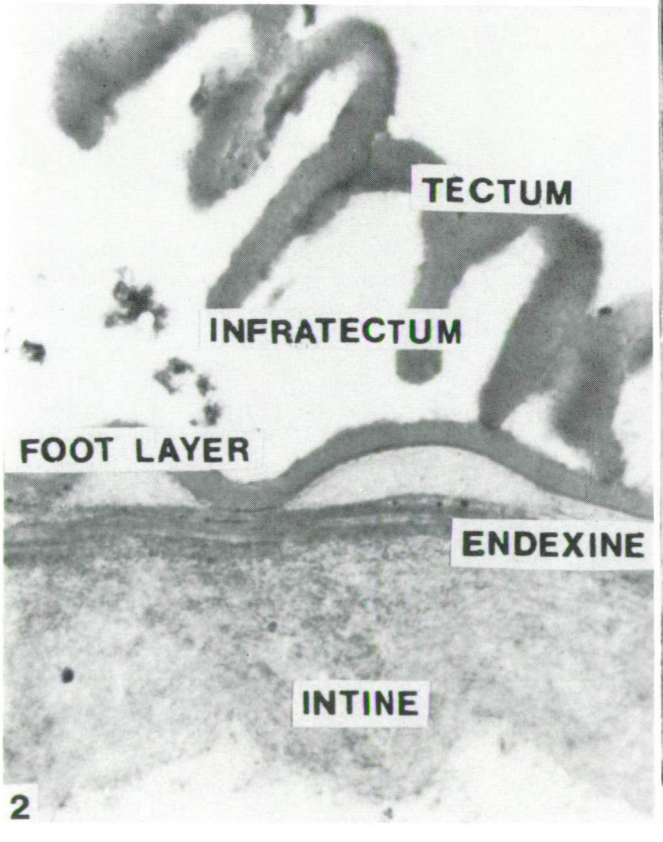
Endexine (Plate 4.5., figs. 1—4)
The inner layers, as it was emphasized in our previous paper (KEDVES et al. 1991, Plate 4.1., fig. 4, p. 33) are complicated. The innermost layer of the foot layer have the strongest electron affinity. This layer is very thin (about 12—15 Å), layer "A" in our previous paper. As a first part of the layers of the endexine this layer is followed by the previously mentioned granular layer; layer "B" in our previous paper. The biopolymer structure of this layer is heterogeneous. There are globular units, with strong electron density interbedded in a faint finely granular substance. The diameter of the granular units is 2—10 Å, mostly 4 Å. The biopolymer organization of this endexine layer is similar to that of the intine. The following layer is more compact than the foot layer, in general it is similar to the ectexine.
Summarizing the above described results, this layer seems to be extremely interesting and peculiar following the treatment regarding its ultrastructure and biopolymer organization.

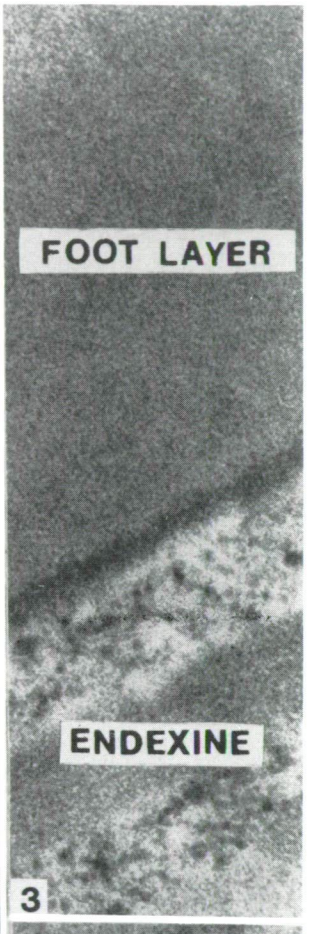
2. THE EFFECT OF THE DIFFERENT SOLVENTS ON ONE SPECIES AND/OR SAMPLE

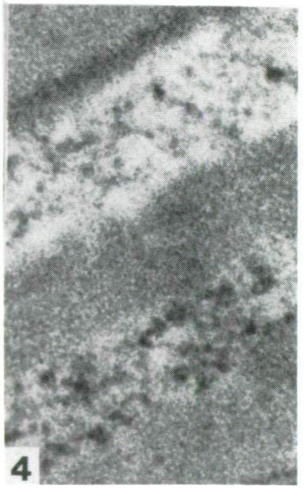
Partial degradation with tetrahydrofuran

Pinus mugo TURRA
Experiment No: 634
(Plate 4. 6., figs. 1—4)


TECTUM
INFRATECTUM
FOOT LAYER
ENDEXINE
INTINE
1


TECTUM
INFRATECTUM
FOOT LAYER
ENDEXINE
INTINE
2


FOOT LAYER
ENDEXINE
3


4

◀ Plate 4. 5.

1—4. *Picea glauca* (MOENCH.) VOSS.
TEM pictures from the pollen grains after partial degradation with diethylether. Experiment No: 680.

1. General survey picture of the wall of the corpus after treatment. Negative no: 525, 25.000x.
2. TEM picture of the wall of the corpus/saccus bordering after degradation. Negative no: 526, 25.000x.

3, 4. Biopolymer organization of the foot layer and the endexine of the corpus. Negative no: 528, 250.000x.

1
OUTER SURFACE
3
FOOT LAYER
ENDEXINE
INTINE
2
INFRATECTUM
FOOT LAYER
4
OUTER SURFACE

This experiment resulted for the first time in the demonstration of the characteristic glycogen molecular chains published earlier in our preliminary short communication (KEDVES et al., 1991, Plate 4. 1., fig. 2, p. 33). The results in this subject are summarized in detail as follows.

Corpus (Plate 4.6., figs. 1—3)

Tectum (Plate 4.6., fig. 1)

The above mentioned glycogen molecular chains are well shown. The diameter of the globular units is 2—10 Å, mostly 4—6 Å. The distance, or better say the "α (1→4) linkage between two glucose units" (cf. DARNELL et al. 1986, p. 99) is about 4—6 Å. We have observed the "α (1→6) linkage between two glucose chains" too, but for the detailed investigations we need further experimental data.

Infratectal and foot layer (Plate 4. 6., fig. 2, 3)

From the infratectal layer, a low magnified picture is presented here. In the highly magnified pictures of this layer, glucose chains or globular, highly organized biopolymer units were not observed. The biopolymer organization of the foot layer, is identic with that of the infratectum (Plate 4. 6., fig. 3).

Endexine and intine (Plate 4. 6., fig. 2, 3)

This layer well separates from the ectexine by its strong electron density. It is an intermediate zone between these two layers. In the endexine sensu stricto no globular biopolymer structures were observed. At the endexine/intine bordering part, howewer, there are globular units of 2—10 Å in diameter, mostly of 6—8 Å. The "distance" between these units is about 2—3 Å. The globular units are sometimes arranged into fibrillar or lamellar structures. The origin of these elements needs further investigations. The globular units of the outer part of the intine are a little smaller; 2—8 Å, mostly 4—6 Å. Distance between these globular units is 2—6 Å, mostly 2—4 Å.

Saccus (Plate 4. 6., fig. 4)

Tectum (Plate 4. 6., fig 4)

On the outer surface of the tectum the glycogen molecular chains are well shown. The diameter of the globular (cf. glucose) units is 2—10 Å, mostly 4—6 Å. The "α (1→4) linkage between two glucose units" is 4—6 Å.

◀ Plate 4.6.

1—4. *Pinus mugo* TURRA.
TEM pictures of the pollen grains after partial degradation with tetrahydrofuran. Experiment No: 634.

1. Glycogen molecular chains of the partially degraded tectum of the corpus. Negative no: 399, 500.000x.
2. TEM picture of the inner layers of the exine after THF treatment. Negative no: 392, 50.000x.
3. Molecular structure of the inner layers of the pollen wall after the treatment. Negative no: 395, 500.000x.
4. Biopolymer structure of the partially degraded tectum of the saccus. The glycogen molecular chains are clearly shown. Negative no: 387, 500.000x.

1
SACCUS
a1
a2
a3
2
TECTUM
OUTER SURFACE
3
4

Infratectum

On the basis of our TEM data the biopolymer units were not suitable for measurements.

The inner exine layers of the saccus were not investigated in detail.

Partial degradation with n-pentane

Pinus mugo TURRA
Experiment No: 656
(Plate 4. 7., figs. 1—4)

In this experiment the saccus was investigated only. The low magnified picture illustrates well the consequence of the n-pentane treatment the ectexine has a strong electron density.

Tectum (Plate 4. 7., fig. 2)

On the outer surface of the tectum, the protective biopolymer layer is not characteristic. The glycogen chains, which were very characteristic and well demonstrated in the previous experiment of the same species were in all probability destroyed during treatment with n-pentane. Less characteristic globular units of 2—10 Å were observed (mostly of 4—8 Å) being probably the remains of the glycogen chains.

Infratectum (Plate 4. 7., fig. 3, 4)

In this ectexine layer the molecular structures (probably glycogen) are better preserved, see fig. 3, in Plate 4—7. The globular units are of 2—10 Å in diameter, mostly 4—10 Å.

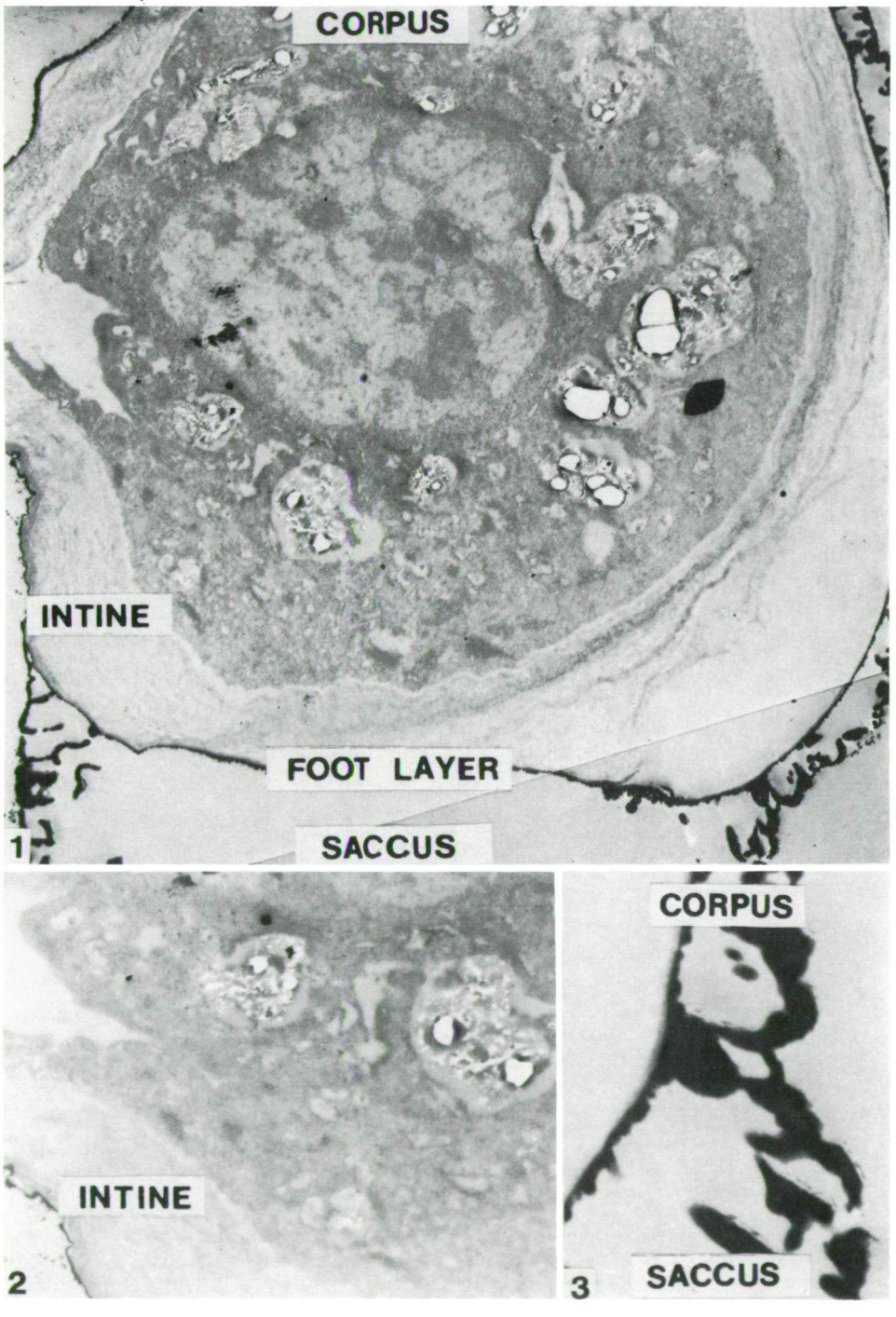
Partial degradation with pyrrolidine

Pinus mugo TURRA
Experiment No: 670
(Plate 4.8., figs. 1—3, plate 4. 9., figs. 1—6)

In the general survey pictures (Plate 4. 8., figs. 1—3) it is well shown that the electron density of the ectexine is very strong after the treatment. The intine is lamellar, and seemingly with secondary alterations. As regards the details, the

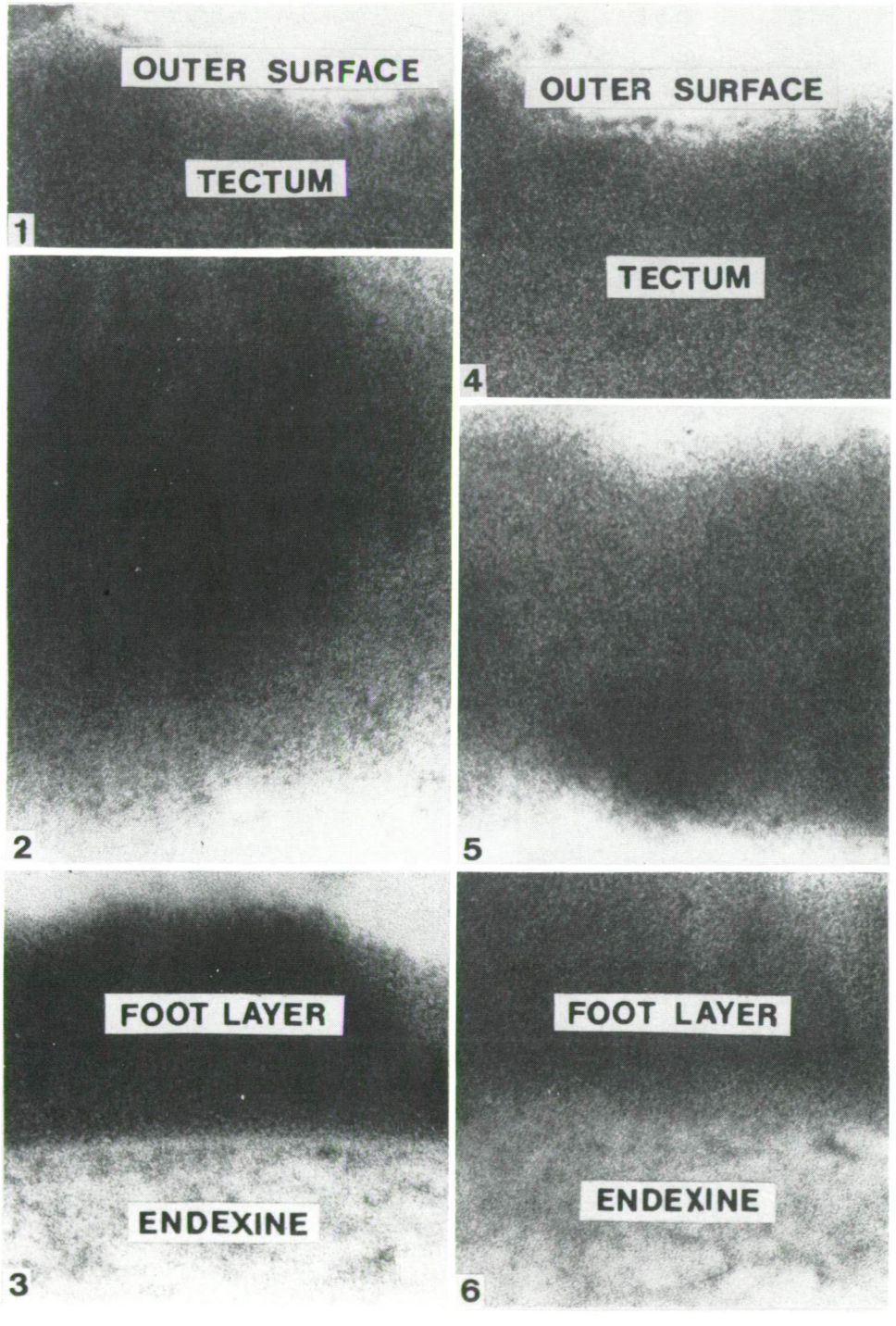
◀ Plate 4. 7.

1—4. *Pinus mugo* TURRA.
TEM pictures of the pollen grains after partial degradation with n-pentane. Experiment No: 656.
1. General survey picture of the outer part of the saccus. Negative no: 748, 5.000x.
2. Biopolymer structure of the partially degraded tectum of the saccus. Negative no: 750, 500.000x.
3, 4. TEM pictures of the partially degraded infratectum. Negative no: 752 and 751 respectively, 500.000x.


CORPUS
INTINE
FOOT LAYER
SACCUS
1
INTINE
2
CORPUS
SACCUS
3

◀ Plate 4.8.

1—3. *Pinus mugo* TURRA.
TEM pictures of the pollen grains after partial degradation with pyrrolidine. Experiment No: 670.
1. General survey picture of the pollen grain after experiment. Negative no: 755 and 756, 2.500x.
2. Part of the corpus in the apertural area. Negative no: 757, 5.000x.
3. Ultrastructure of the corpus/saccus border region. Negative no: 764, 10.000x.


OUTER SURFACE
TECTUM
1
2
OUTER SURFACE
TECTUM
4
5
FOOT LAYER
ENDEXINE
3
FOOT LAYER
ENDEXINE
6

following can be emphasized. In general, the biopolymer structure, firstly the surface protecting biopolymer system is damaged.

Corpus (Plate 4.9., figs. 1—3)

Tectum (Plate 4. 9., fig. 1)

The more or less damaged unilayered protective biopolymer system is also composed of globular units of 2—10 Å in diameter, mostly of 4—6 Å.

Infratectum (Plate 4.9., fig. 2)

On the surfaces of this layer only very damaged biopolymer structures were observed. The preservation of these units is insufficient for exact measurements.

Foot layer and endexine (Plate 4.9., fig. 3)

Taking into consideration the results of the previous experiment, it is interesting that the endexine was strongly dissolved, and its electron density is extremely low compared to the foot layer. Damaged globular units of 3—8 Å in diameter were observed which form filaments or lamellae, or irregular network system.

Saccus (Plate 4.9., figs. 4—6)

Tectum (Plate 4.9., fig. 4)

Similar to those of the corpus, the surface protective layer is composed of globular units of 3—10 Å in diameter, mostly of 4—10 Å.

Infratectum (Plate 4.9., fig. 5)

On the surface of this layer no highly organized biopolymer structures were observed.

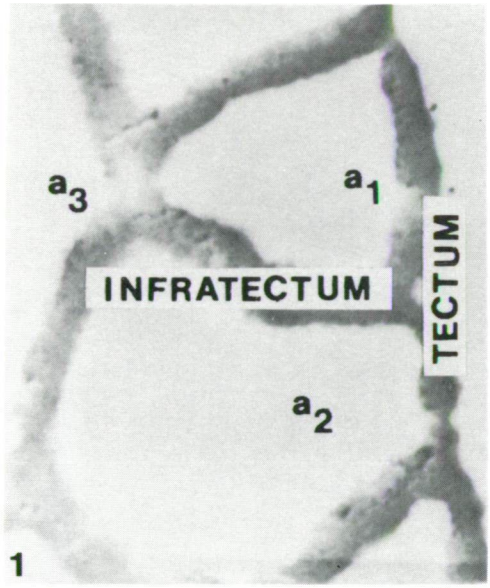
Foot layer and endexine (Plate 4.9., fig. 6)

Beneath the relatively electron dense foot layer, the endexine is a little more damaged than those of the corpus.

◀ Plate 4.9.

1—6. *Pinus mugo* TURRA.
Biopolymer structure of the exine of the pollen grains after partial degradation with pyrrolidine. Experiment No: 670.

1. Detail of the tectum of the corpus. Negative no: 765, 250.000x.
2. Partially degraded infratectum of the corpus. Negative no: 766, 250.000x.
3. Biopolymer structure of the inner layers of the exine after treatment. Negative no: 767, 250.000x.
4. Tectum surface of the saccus after treatment. Negative no: 759, 250.000x.
5. TEM picture of the partially degraded infratectum of the saccus. Negative no: 761, 250.000x.
6. Detail of the biopolymer structure of the foot layer and the endexine of the saccus. Negative no: 762, 250.000x.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INFRATECTUM
TECTUM
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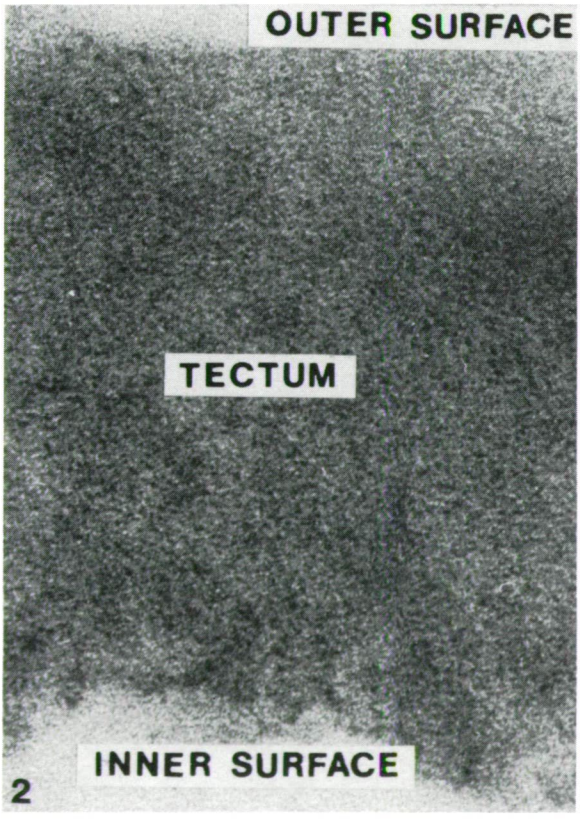
1


3


OUTER SURFACE
TECTUM
INNER SURFACE
2


4

◀ Plate 4. 10.

1—4. *Pinus silvestris* L.
Biopolymer structure of the pollen grains after partial degradation with n-pentane. Experiment No: 657.
1. Detail of the saccus after treatment. Negative no: 512, 25.000x.
2. Biopolymer structure of the partially degraded tectum of the saccus. Negative no: 513, 500.000x.
3. Detail of the infratectal layer after treatment. Negative no: 514, 100.000x.
4. Highly magnified part of the infratectal layer. Negative no: 515, Magnification 1 million.

3. VARIA

Partial degradation with n-pentane

Pinus silvestris L.
Experiment No: 513
(Plate 4.10., figs. 1—4)

In this experiment only the saccus was investigated with the TEM method. The strong degradation of the ectexine is well shown in the low magnified picture (Plate 4. 10., fig. 1).

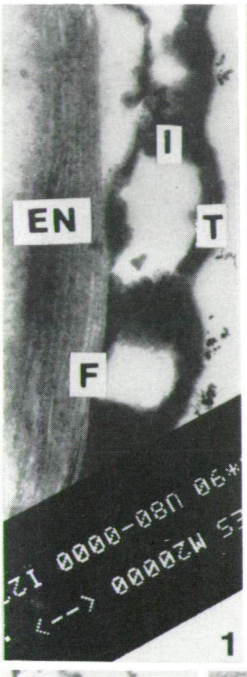
Tectum (Plate 4.10., fig. 2)
The protective biopolymer units of the surface are damaged. In the outer surface globular units of 2—6 Å (mostly 2—4 Å) diameter were observed. The measured units of the inner surface are of 4—10 Å in diameter, mostly 6—8 Å. This difference in size of these units may be the consequence of the extensive degradation.

Infratectum (Plate 4.10., fig. 3, 4)
On the surface of this layer no molecular structure was observed. The superficial degradation of the biopolymer system can be clearly seen. Only the remnants of the protective biopolymer layer were observed.

Partial degradation with pyrrolidine

Pinus griffithii McClell
Experiment No: 668
(Plate 4. 11., figs. 1—4)

The strong electron density of the ectexine layers is well shown in the low magnified pictures (Plate 4.11., fig. 1, 2). It is worth of mentioning that the dissolution of the lamellar endexine is not uniform. In fig. 1 of Plate 4.10., the lamellar endexine is well shown. The disappearance of this layer is illustrated in Plate 4.11., fig. 2. At this experiment the corpus was investigated only by the TEM method.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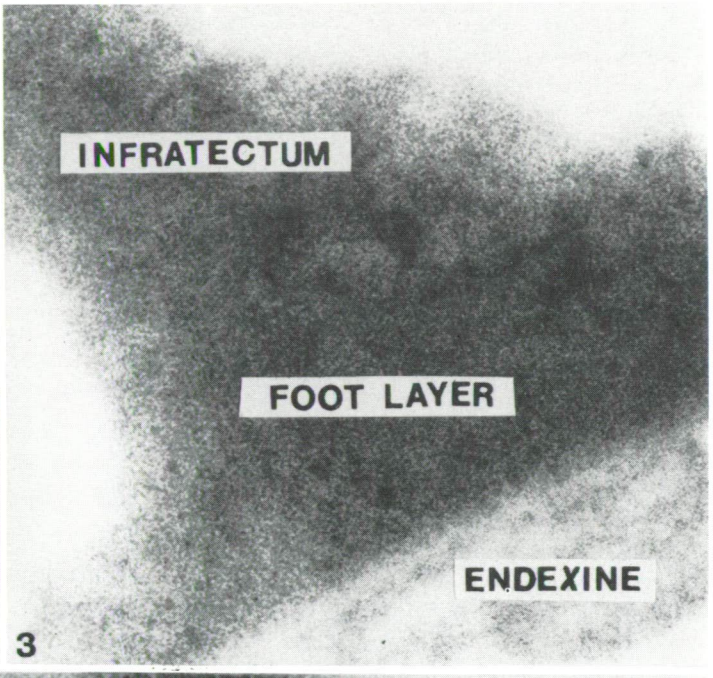
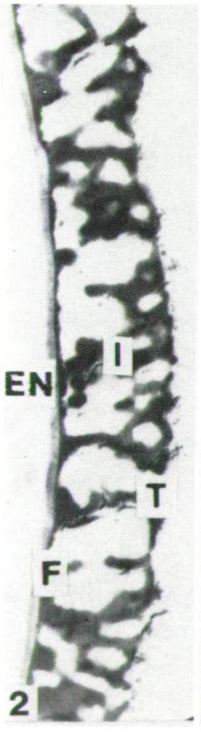

INFRATECTUM
FOOT LAYER
ENDEXINE
3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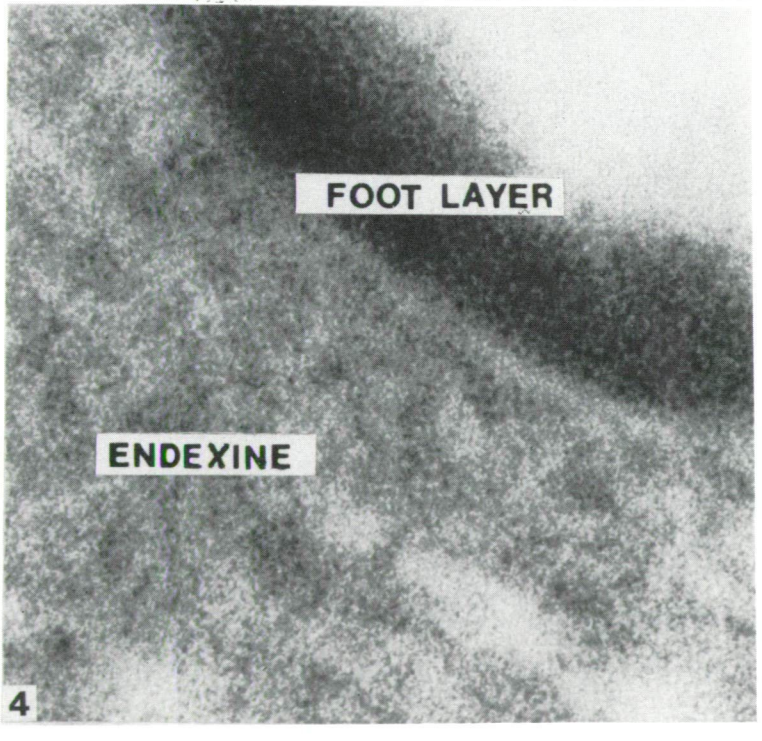

FOOT LAYER
ENDEXINE
4

Plate 4.11.

1—4. *Pinus griffithii* McCLELL.
TEM picture of the partially degraded pollen grains with pyrrolidine. Experiment No: 669.
1, 2. General survey pictures demonstrate the exine stratification of the corpus after degradation.
1. Negative no: 437, 25.000x.
2. Negative no: 438, 10.000x.
3. Biopolymer organization of the inner layers of the corpus. Negative no: 440, 200.000x.
4. Biopolymer structure of the foot layer and the endexine. Negative no: 434, 500.000x.

Tectum (not illustrated)

On the surface, extremely damaged globular biopolymer units were observed. Diameter of these units is between 2—10 Å, mostly of 4—6 Å.

Infratectum, foot layer and endexine (Plate 4.11., fig. 3, 4)

The small stabilizing units are well shown. The highly organized globular structures however are not so characteristic. Their diameter is between 2—10 Å, mostly of 4—8 Å. These values are measured from the endexine. The difference in the electron density of the foot layer and the endexine is very characteristic.

Partial degradation with pyrrolidine

Picea glauca (MOENCH.) VOSS.
Experiment No: 671
(Plate 4.12., figs. 1—4)

Corpus (Plate 4.12., fig. 1, 2)

In the low magnified picture, the strong electron density of the ectexine and the degradation of the endexine and the intine are well illustrated.

Tectum (not illustrated in high magnification)

On the surface, damaged globular units were observed, as the remnants of the protective molecular layer. The diameter of these globular elements is 2—10 Å, mostly 4—6 Å.

Infratectum (Plate 4.12., fig. 2)

The biopolymer structure of this layer is identical with that of the tectum.

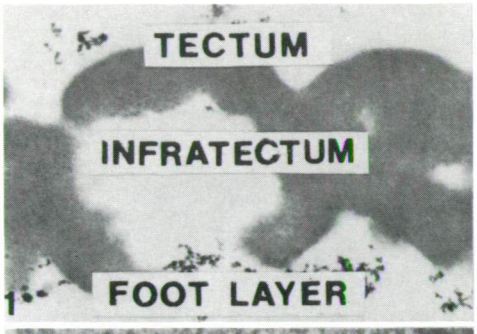
Foot layer (not illustrated)

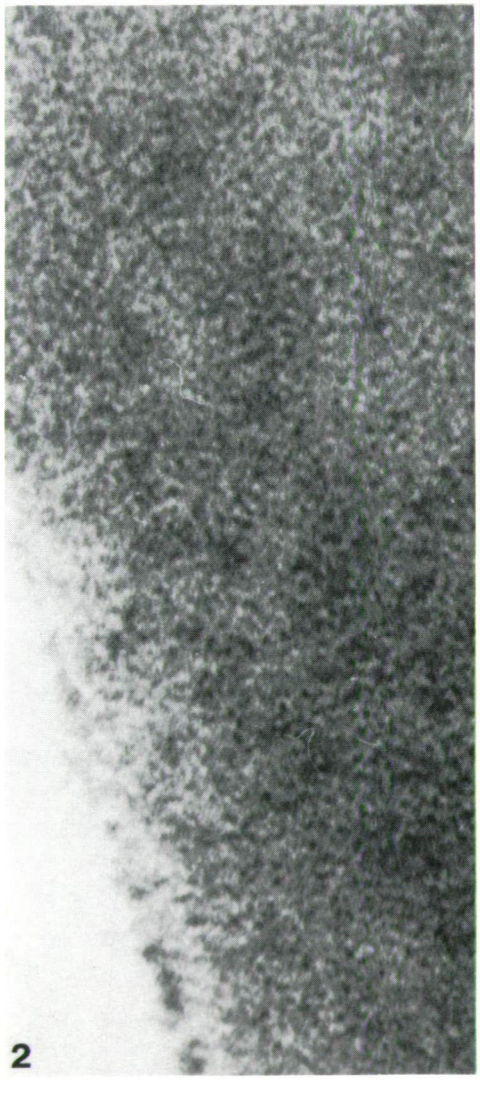
In the foot layer, and probably in the endexine/or intine border larger globular elements were observed, they are not so well preserved. The electron density is very strong, the diameter is 8—20 Å, mostly 14—20 Å.

Saccus (Plate 4.12., fig. 3, 4)

Tectum (Plate 4.12., fig. 4)

The different kinds of alveoli are illustrated in fig. 3, Plate 4.12. The globular


TECTUM
INFRATECTUM
FOOT LAYER
1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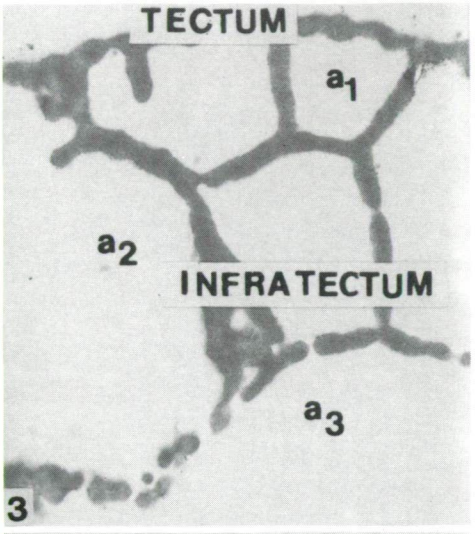
TECTUM
a1
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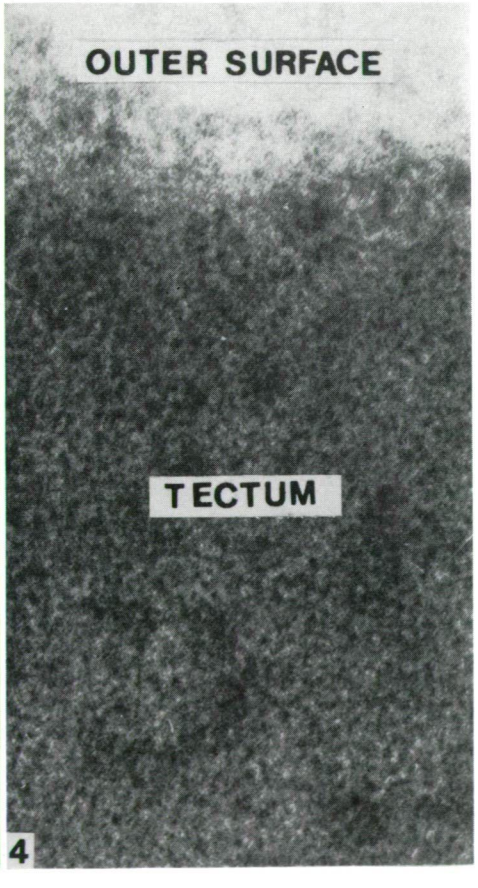
INFRATECTUM
a3
3


OUTER SURFACE
TECTUM
4

molecular remnants are of 2—8 Å in diameter, mostly of 3—6 Å. These structures are extremely damaged.

Infratectum (not illustrated)

The damaged biopolymer units are of 2—8 Å in diameter, mostly of 4—6 Å. The further inner layers were not investigated in this respect.

◀ Plate 4.12.

1—4. *Picea glauca* (MOENCH.) VOSS.
Partially degraded pollen grains with pyrrolidine. Experiment No: 671.

1. Detail of the exine stratification of the corpus after treatment. Negative no: 516, 25.000x.
2. Biopolymer organization of the partially degraded infratectum of the corpus. Negative no: 519, 500.000x.
3. Detail from the ultrastructure of the tectum after degradation. Negative no: 522, 10.000x.
4. Biopolymer structure of the tectum after pyrrolidine treatment. Negative no: 523, 500.000x.

Discussion and Conclusions

1. The effect of the diethylether on all of the saccate pollen grains investigated can be summarized as follows.

1.1. This solvent is particularly suitable for the investigation of the biopolymer and/or molecular structure of the inner wall layers of the saccate gymnosperm pollen grains. Foot layer, endexine, the different layers of the intine can be investigated. Its effect on the intine, as it is well illustrated in Plate 4.1., fig. 1. (*Pinus mugo* TURRA) is similar to the effect on the pollen grains of *Corylus avellana* L. of the experiment C-2A (20 mg air dried pollen grains+2 ml *Helix* enzyme 2%,+1 ml merkapto-ethanol, temperature 30 °C, length of time 5^h, cf. KEDVES, 1986).

1.2. The globular biopolymer units — probably the glucose chains on the surfaces are in general damaged, this is the first sign of the degradation of the tectum.

2. We have only one data about the effect of the tetrahydrofuran on the exine of the bisaccate gymnosperm pollen grains. This seems the best solvent to demonstrate the glycogene chains of the surface. This method with this solvent may be a basis for another research program of different taxa of sporomorphs and different kinds of plant cell walls.

3. The n-pentane dissolved the protective biopolymer system of the surfaces. The strong electron density of the ectexine layers indicates important changes inside the exine. The dissolution of the quasi-crystalloid skeleton may be presumed.

4. Pyrrolidine seems to be the best to dissolve the quasi-crystalloid biopolymer skeleton. On the basis of our up-to-date knowledge by the dissapearance of the surface protecting layer or in general the highly organized units we can presume that in the greatest part the stabilizing biopolymer system is present. High magnified negatives, as 250.000x and 400.000x seems to be suitable to give sufficient data for the investigation of the molecular organization of the stabilizing system of the quasi-crystalloid biopolymer skeleton. The detailed investigation and evaluation of

the pictures of high magnification (2.5 and 5 million) will be the subject of further investigations. We hope that on the basis of these data we will have the opportunity to start the combined modelling of the biopolymer structure of the sporoderm.

Acknowledgements

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5. THREE DIMENSIONAL MODELLING OF THE BIOPOLYMER STRUCTURE OF THE PLANT CELL WALL II.

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Abstract

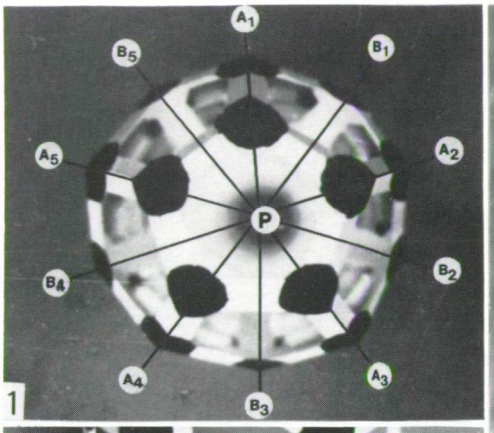
On the basis of the elementary (basic) units of the structural highly organized biopolymer skeleton the following types were distinguished: 1. Primary units; filaments, lamellae, helical, microtubular, globular (PENROSE-like) highly organized biopolymer skeleton structural elements. The basic (building) unit is a pentagonal dodecahedron elementary unit. 2. Secondary units; filaments and the further above mentioned elements of the plant cell wall and the cytoskeleton, but composed from PENROSE-I type biopolymer skeleton. In this way the building unit is composed of 13 pentagonal dodecahedron elementary (basic) units. This paper summarizes the results of the modelling as follows. 1. Filaments including the primary and the secondary biopolymer skeleton. 2. PENROSE-I and PENROSE-II units. 3. Helical (microtubular) secondary unit. 4. Primary modelling of the lamellar system. 5. Particular attempt was bestow upon the biopolymer skeleton of the surface. It was established that quasi-periodic units in linear or two dimensional systems can be periodic, too.

Key words: Plant cell, biopolymer organization, three-dimensional modelling.

Introduction

As it was emphasized in the previous paper (KEDVES, 1991b) the three-dimensional modelling of the quasi-crystalloid biopolymer skeleton will be continued. The first models presented, and discussed in the above mentioned paper were prepared following the most important problems which arose in our last two-dimensional modelling (KEDVES, 1991a; KEDVES and FARKAS, 1991), and the new TEM data concerning the partially degraded plant cell walls. It is clear that our knowledge about the three-dimensional structure of the quasi-crystalloid biopolymer skeleton can not be taken as completely accomplished. But we hope that now we have enough data to have notions concerning this complicated biopolymer system of the most important cellular elements. Besides the highly organized biopolymer structures, particular attention was payed for the surface and/or for the bordering layers of the cellular organells.

On the basis of our up-to-date knowledge about the quasi-crystalloid skeleton of the plant cell wall, and of the newest experimental results we have the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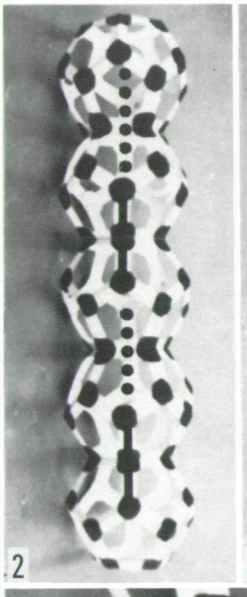
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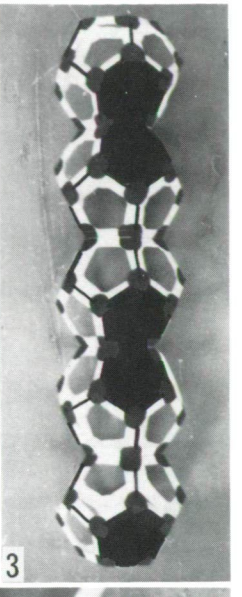
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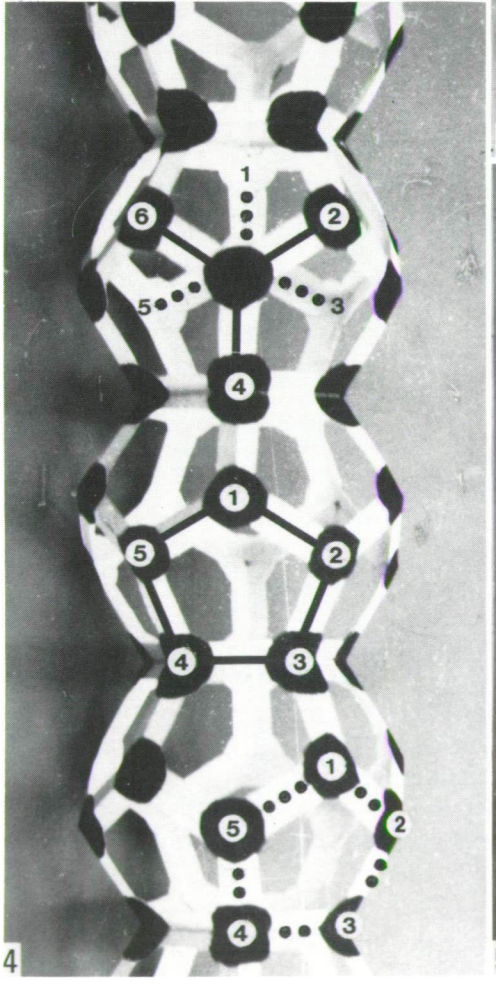
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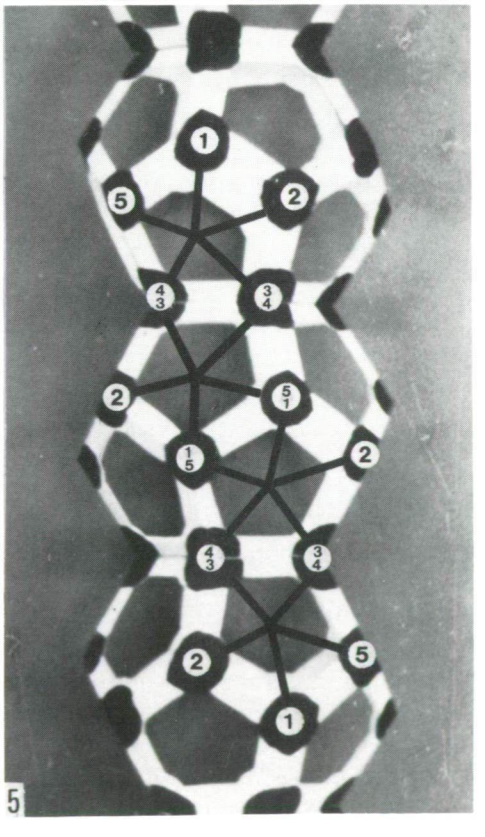
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opportunity to start the combined modelling, which contains two important fields:

i. Investigation of the detailed quasi-crystalloid skeleton.

ii. The modelling of the stabilizing biopolymer system. This latter is also heterogeneous in character. The detailed study of the stabilizing molecular system including its modelling will be the subject of further investigations.

◀ Plate 5.1.

1—5. Quasi-crystalloid skeleton model of the primary filament.
1. Aspect of the quasi-crystalloid skeleton model. In this view the picture is similar to one pentagonal dodecahedrane model. "P" is in the axe of the filament. AP_{1-5} and BP_{1-5} are the axes of the primary rotations.
2,3. Two aspect of first importance of the primary filament.
2. Periodic lateral short axis view. The so-called proximal and distal short axes are in the same plane.
3. Discontinous short axis view. Short axes alternate with two planes of the dodecahedrane.
4,5. Details from the above mentioned two aspects of first importance of the model of the primary filament.
4. The important points of symmetries, or biopolymer units (globular) of the model are indicated. The two times three-fold symmetry can be pointed out.
5. Five-fold points of symmetries of the alternating pentagonal planes.

Methods

The basic — building elementary — units are as it was published in the previous part of this series of papers. Some minor alterations were introduced only as follows.

i. The central biopolymer unit of the PENROSE-I model is no more compact. It is identical with the basic pentagon dodecahedrane unit.

ii. Some alterations were introduced in the colouring of the modelling units to better understand the characteristic features of the biopolymer skeleton, and its points of symmetries in the space.

Results

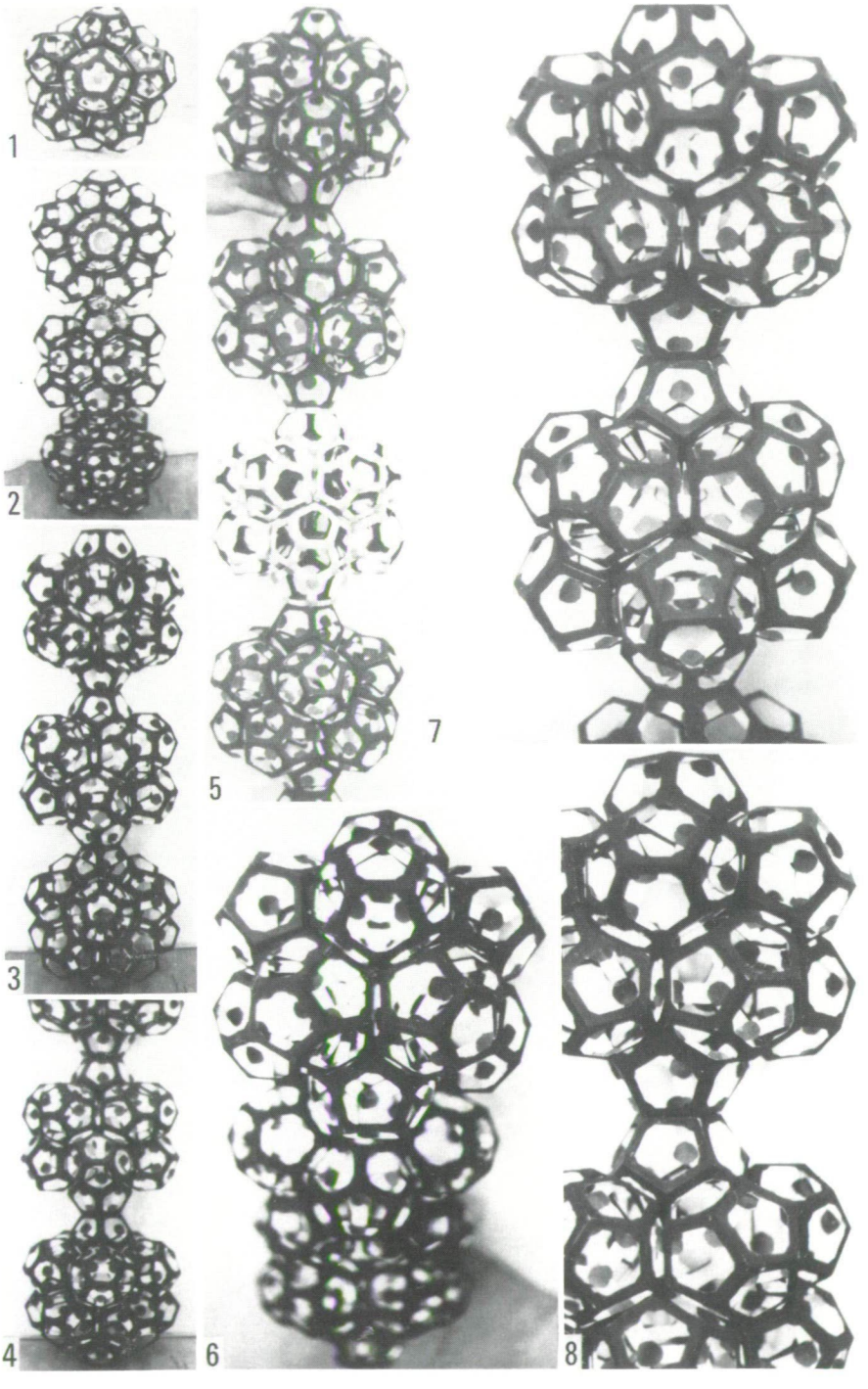
FILAMENTS

Primary (simple) filaments (Plate 5.1., figs. 1—5)

The most important characteristic features of this kind of highly organized biopolymer unit are as follows.

The linear arranged dodecahedron basic skeleton units are connected by five edges, or better to say with one plane of each dodecahedron. In this way there are no frustrations between the building units of this kind of biopolymer skeleton, and it is a peculiar periodical arrangement. At this kind of periodical system two dodecahedron basic skeleton represents one unit (Plate 5.1., fig. 2,3, resp. 4,5).

The three-dimensional quasi-crystalloid units arranged in one linear system accomplish a periodic skeleton.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◀ Plate 5.2.

1—8. First type of connections of the secondary (complex) filament.

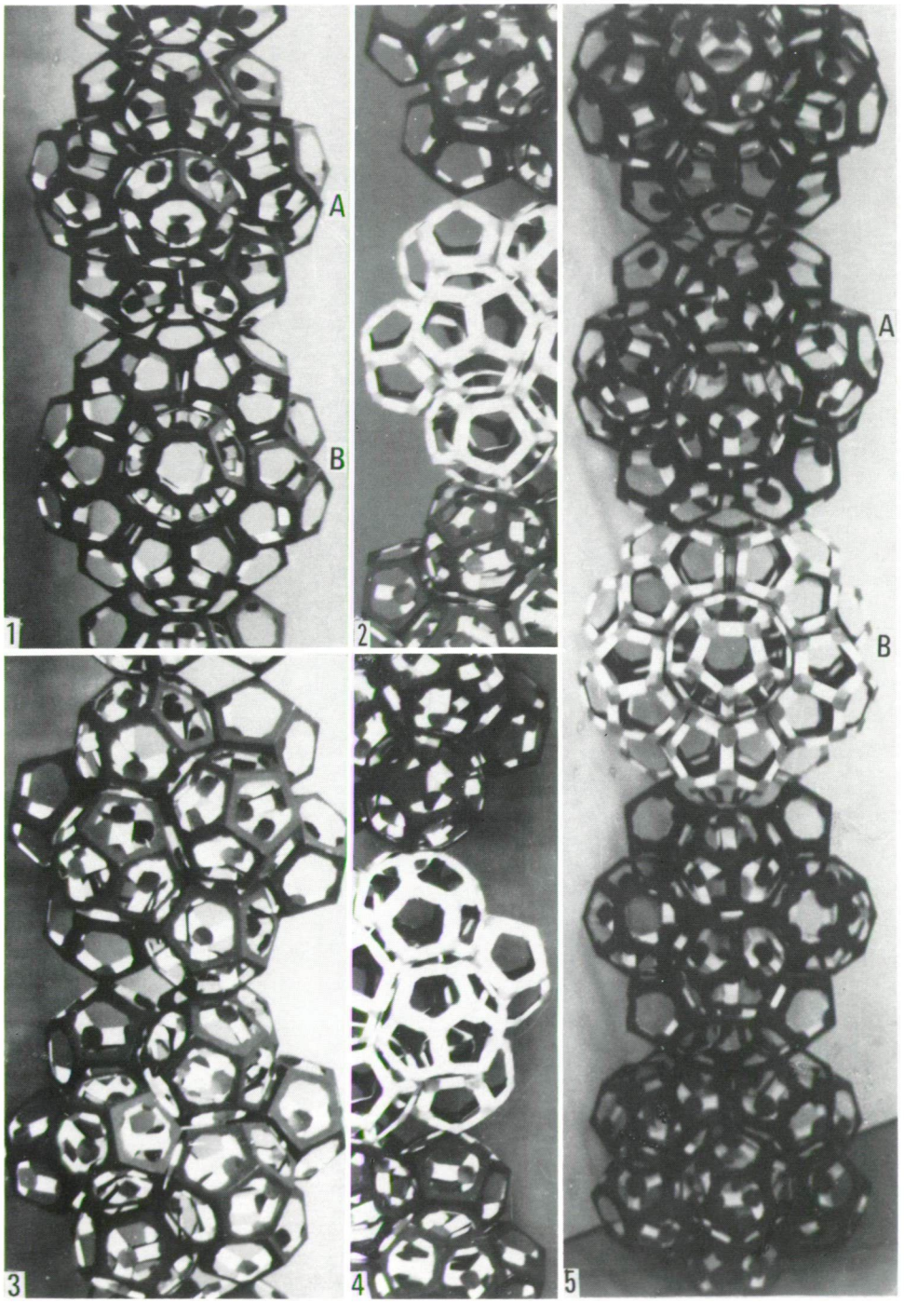
1. Aspect of the quasi-crystalloid skeleton model of the first type of connecting the secondary filament. In this view the picture of this kind of model of quasi-crystalloid filament is similar to one PENROSE—I quasi-crystalloid unit. The regular pentagon in the middle is identical in the point of view of the rotation axes with those illustrated in Plate 5.1., fig. 1.

2—4. Homogeneous complex filament from different views. The elementary PENROSE—I units from these views can be pentagonal or hexagonal.

5. Lateral view of the model of a heterogeneous filament.

6. Oblique lateral view of a homogeneous secondary filament. It is noteworthy that the connections between the PENROSE—I building units are discernible.

7, 8. Magnified pictures from the connecting units of a homogeneous secondary filament from somewhat different lateral views. The alternation of the position of the points of symmetries is interesting and important.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The aspect of the filament (Plate 5.1., fig. 1) well illustrates essentially the symmetries of the quasi-crystalloid basic units (cf. Plate 7.1., fig. 1, KEDVES 1991b, p. 68). In the pictures taken from the lateral views of the model of the filaments the above mentioned periodic characteristic features can be well studied. It seems that there are a lot of opportunities for the investigation of the aspects, two most important views are represented as follows.

1. Periodic lateral short axis view (Plate 5.1., fig. 2,4)

The plane of the short axes is identical with the plane crossing the B_5-P-A_1 line. The different views well represent the different aspects of the pentagonal units. But two times three-fold points of symmetries can also be occur in consequence of the particular superposition of the edges of the pentagon dodecahedrane unit (Plate 5.1., fig. 4).

2. Short axes with planes alternate views (Plate 5.1., fig. 3,5)

In contrast to the previous aspect it is not an axis view. Fig. 3 in Plate 5.1., illustrates well that the short axes end at the apices of a peculiar octahedron composed of two sides of two connected pentagon dodecahedrane units. Fig. 5, of Plate 5.1., illustrates well the connecting globular units, and the AP axes of one unit composed of four pentagons. It is clearly shown that there are differences in the two middle planes in contrast to the first and the fourth pentagon. In this case, the essential differences came from the 5—5 connecting globular units of two pentagonal sides. This plane represents the first circle of points of symmetries composed of 5 points, cf. A_1, A_2, A_3, A_4 and A_5 of fig. 1, Plate 10.1. The lateral zigzag, in the view of the filament represents the second circle, composed of 10 points of symmetries of the edges, cf. A_1, B_1, A_2, B_2, A_3, B_3, A_4, B_4, A_5, B_5, A_6, B_6, A_7, B_7, A_8, B_8, A_9, B_9, A_{10}, and B_{10} of fig. 1, Plate 5.1.

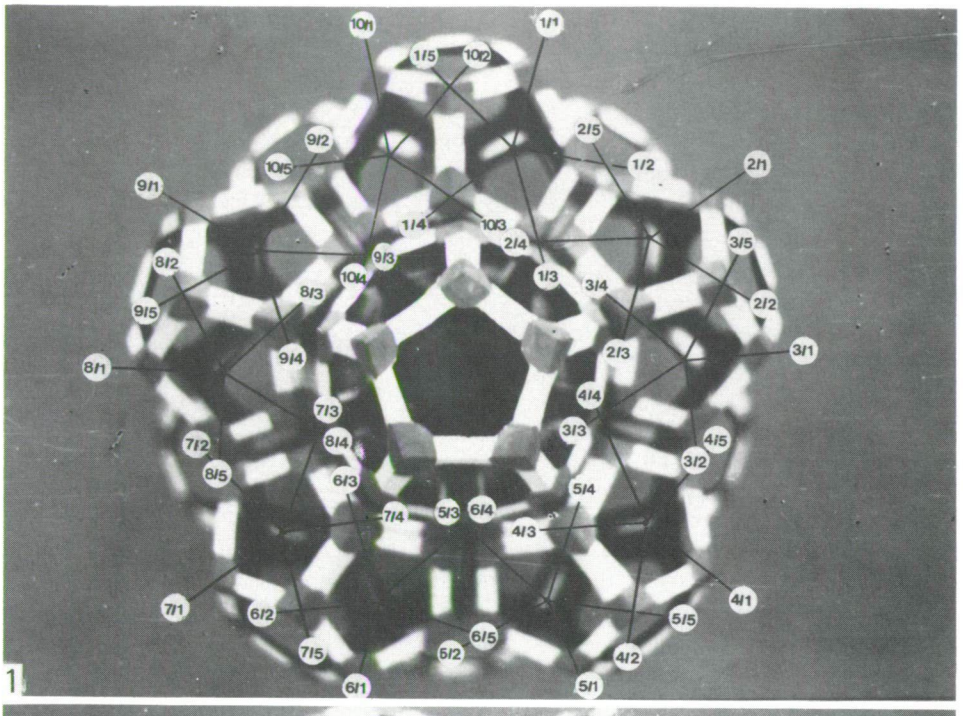
Secondary (complex) filaments
(Plate 5.2., figs. 1—8., plate 5.3., figs. 1—5)

In this case the building elements are the PENROSE-I units. Based on our up-to-date knowledge four secondary filament types have been distinguished, on the basis of two points of views, as follows.

i. The building PENROSE-I units may be homogeneous or heterogeneous.

◀ Plate 5.3.

1—5. Second type of connecting the secondary (complex) filament.
1, 3. Homogeneous complex filament from two important views.
1. The building PENROSE—I units are in two kinds of symmetry position; A=The contour is hexagonal, in the centre a triangular symmetry is characteristic. B=Pentagonal contour in the centre with basic regular pentagonal polygon.
3. The lateral view; the connecting apices are in the middle of the filament.
2,4. Detail from the lateral part of the heterogeneous complex filament.
5. General aspect of the heterogeneous complex filament. This view corresponds to those represented in fig. 1, is perpendicular to the middle point of one connecting side.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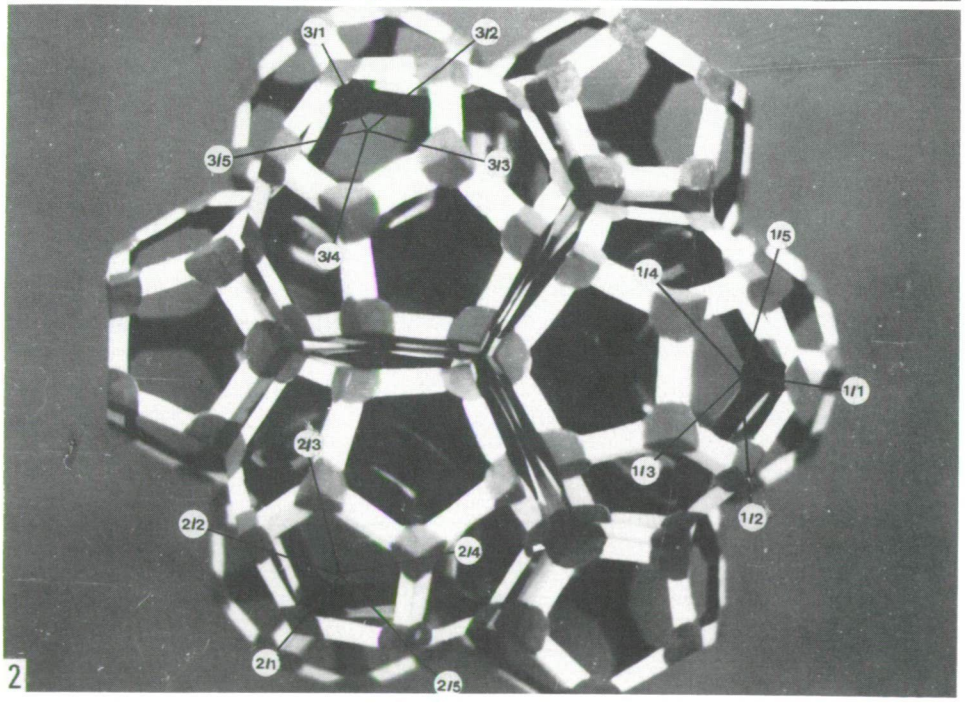
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Concerning this problem it is necessary to emphasize that at this kind of filaments, at this moment it is hypothetical that different kinds of molecular structures can occur in the same quasi-crystalloid biopolymer skeleton system (Plate 5.2., fig. 5., plate 5.3., fig. 2, 4, 5).

ii. Following the connecting points of symmetries, further two major types can be distinguished:

Five points of symmetries, respectively one plane of each PENROSE-I unit (Plate 5.2., figs. 1—8) are connected. In this case the axe of this secondary filament is essentially identical with those of the primary (simple) filament with the above mentioned periodicity. Each PENROSE-I unit contributes with three basic pentagon dodecahedrae. These are in the axis of this unit. The alternation of the PENROSE-I unit is identical with the two principal aspects of the PENROSE-I model, illustrated in the 5.4., fig. 1,2.

The second connection type is the so-called PENROSE-II. In this case three times one side, or two times six points of symmetries are connected. In the previous paper the frustration between 8 points of symmetries (or globular biopolymer units) was emphasized (KEDVES 1991b, p. 70, fig. 1).

The axe of this kind of filament is in the middle of three pentagonal polygon sides. This characteristic feature completely differs from the above mentioned ones. The previously established axes which across the centrum of two opposite pentagonal polygon units is a form of zigzag. The angles of these axis parts are 60°. It is an interesting alternate periodicity, in this case there are PENROSE-I type units, having such axis too, which are completely at the right angles to the composite filament axis (B). It is self understanding, that there are a lot of further axes.

DIFFERENT ORGANIZATION LEVELS OF THE PENROSE-UNITS

Penrose-I type biopolymer skeleton (Plate 5.4., fig. 1,2)

As it was emphasized previously, this kind of biopolymer model was prepared and discussed in the first part of this series of papers, but in a little different manner, the central pentagon dodecahedron was compact. Moreover an attempt was made to start the investigation of the PENROSE-II quasi-crystalloid model (cf. KEDVES 1991b, Plate 7.2., fig. 1,2, p. 70).

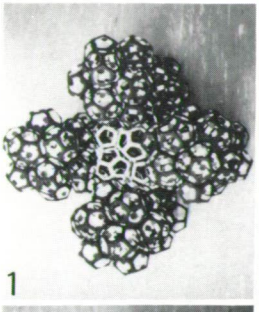
This unit is extremely important in the further organization levels of the biopolymer skeleton, as the "basic unit". This was pointed out previously, too, in

◀ Plate 5.4.

1,2. Two major views in the symmetry of the PENROSE—I quasi-crystalloid biopolymer skeleton.

1. Quasi-periodic view of the PENROSE—I skeleton. The contour is pentagonal. The primary rotation axes are indicated on the outer ("second circle") of pentagonal polygons.

2. The PENROSE—II connection view of the same PENROSE—I model. The contour is hexagonal, and no central regular pentagon is in the centre.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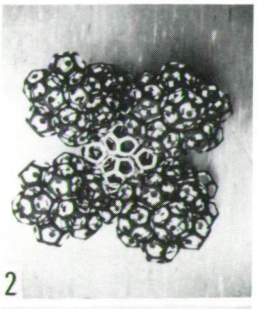
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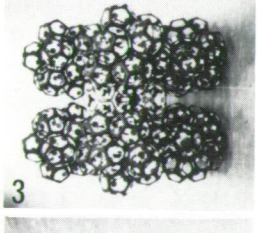
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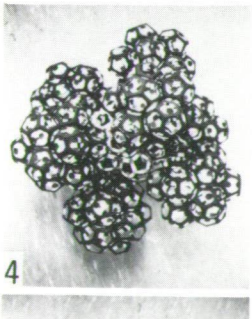
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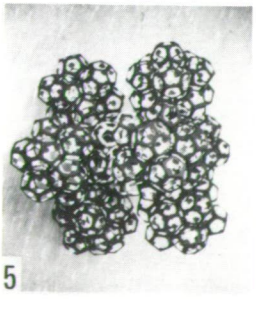
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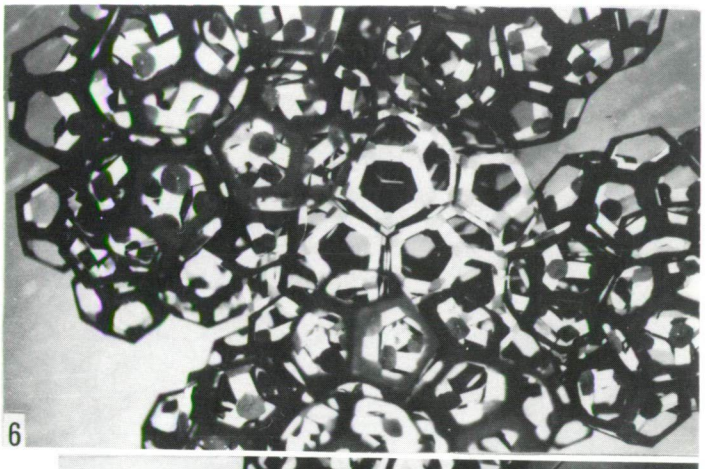
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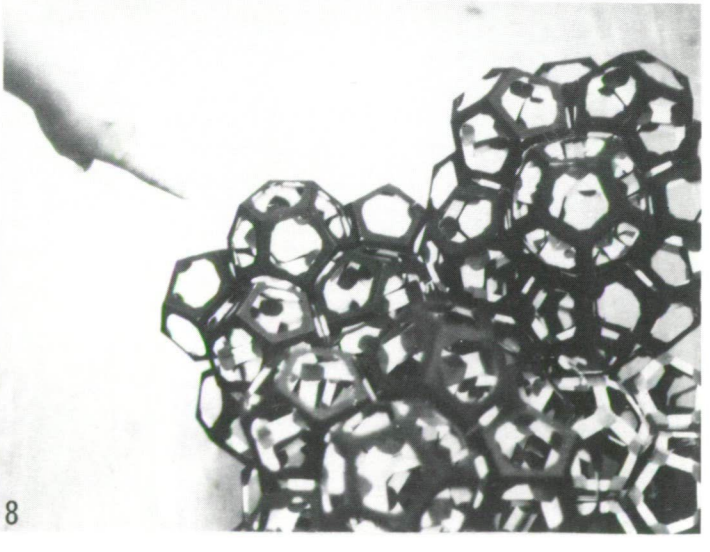
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◀ Plate 5.5.

1—8. Modelling of the PENROSE—II biopolymer skeleton.
1—7. First step of the modelling of the PENROSE—II biopolymer skeleton. The light, central PENROSE—I skeleton is surrounded with eight further, "surrounding PENROSE—I" units. The connection between these PENROSE—I units is the so-called classical PENROSE—II connection; two times three sides (12 edges, or globular biopolymer units of the pentagons).
1—5. The first step in the building of the PENROSE—II biopolymer skeleton model from different aspects.
6,7. Detail from the central and the surrounding PENROSE—I biopolymer units. The great number of points of symmetries is noteworthy.
8. One PENROSE—I biopolymer unit, connected with the second step type connection. At this kind of connection the surrounding PENROSE—I skeleton does not reach the central PENROSE—I biopolymer unit. The connections are with the "first step connected" surrounding biopolymer units. The left hand of Mrs. BIRÓ—HALÁSZ indicates this unit.

connection with the organization systems of the filaments, it is necessary to add some methodical establishments, as follows.

i. There are two major views in the symmetries (Plate 5.4., fig. 1,2)

At the first one, the view axis is at the right angle to the plane of one pentagonal side. In this way, the concentric points of symmetries are well shown. The probable rotation axes are indicated at the "second circle" of pentagons. As it was very characteristic at the quasi-crystalloid skeleton of the filaments, the AP axis is a constant line.

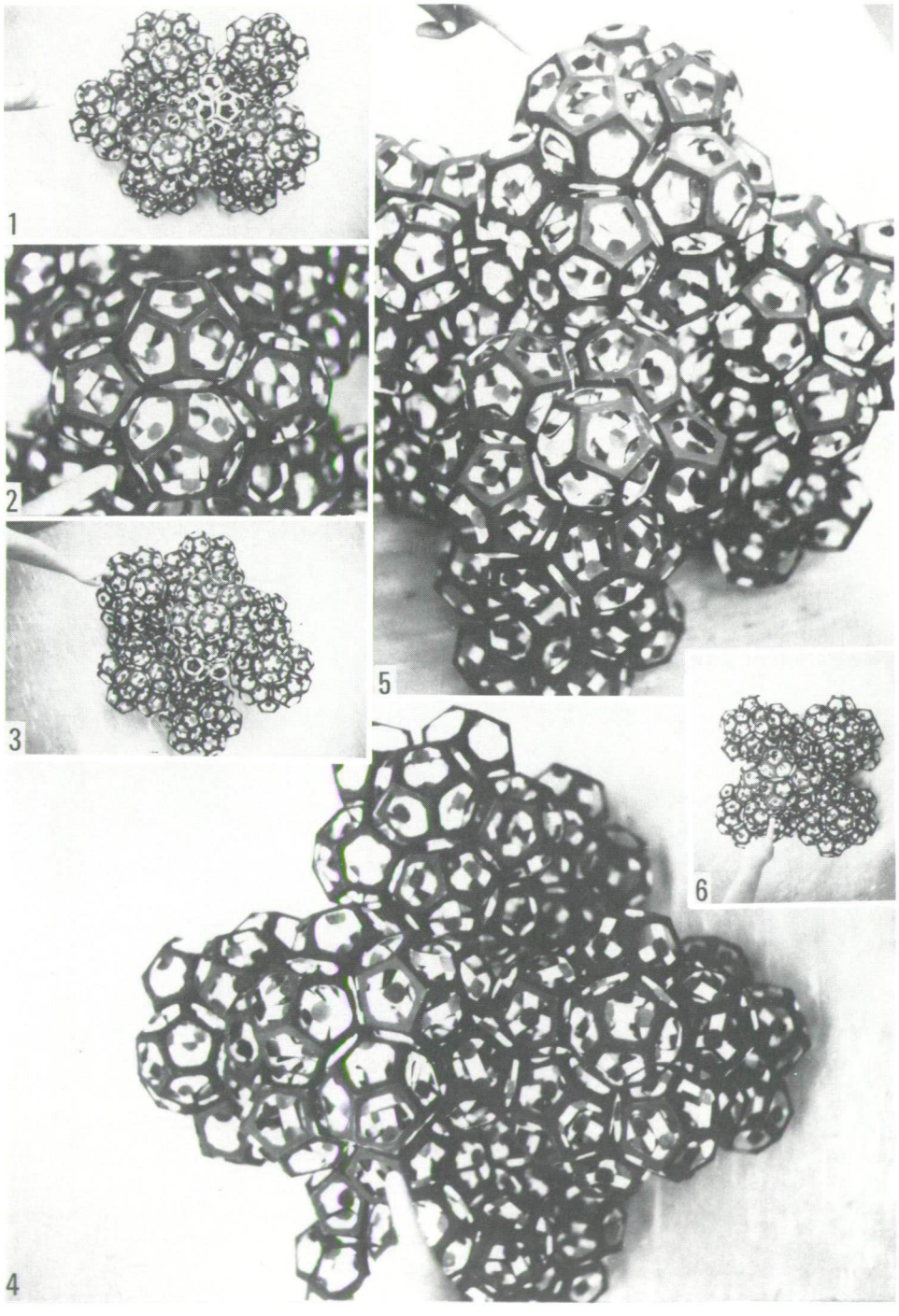
It is important that the contour of the PENROSE—I unit from this view is a regular pentagon, in this way essentially this is a quasi-periodic system.

ii. The same model in the so-called PENROSE—II connection view (six points, or three sides) have a hexagonal contour, so this seems to be a periodic unit. No central pentagon, the axis of this unit is the middle points of three apices of pentagonal sides. The rotation axes of three pentagonal polygon sides are indicated. This is also for the better understanding and interpretation of our TEM pictures of the partially degraded plant cell walls, and the results of its rotations.

Penrose—II biopolymer unit
(Plate 5.5., figs. 1—8, plate 5.6., figs. 1—6)

To better understand this complicated model the coloration of the central PENROSE—I skeleton was prepared in a different manner; the lighter skeleton. After starting the building of the complete "big PENROSE—II" biopolymer skeleton model we observed as follows.

Eight „surrounding PENROSE—I" units can be connected to the central unit, with the so-called classical connection, two times three sides (12 edges, or globular biopolymer units of the pentagons). From different views this, not complete PENROSE—II biopolymer skeleton have tetragonal, pentagonal, or hexagonal ambitus. The diameter of the "tetragonal view" is about 144 Å, the maximum size of the hexagonal one is 154 Å (Plate 5.5., figs. 1—5). Fig. 6 and 7 illustrate well the really complicated points of symmetry system around the central PENROSE—I skeleton from different views, and the large holes between the "surrounding" or "satellite" PENROSE—I system.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◀ Plate 5.6.

1–6. The first step connected PENROSE–II biopolymer model with one second type connected PENROSE–I unit. The pictures were taken from different positions and magnifications. The "second step connected" PENROSE–I unit is indicated by Mrs. BIRÓ–HALÁSZ. It is noteworthy that without indication it is almost impossible to observe the differences between the two kind connected surrounding PENROSE–I units.

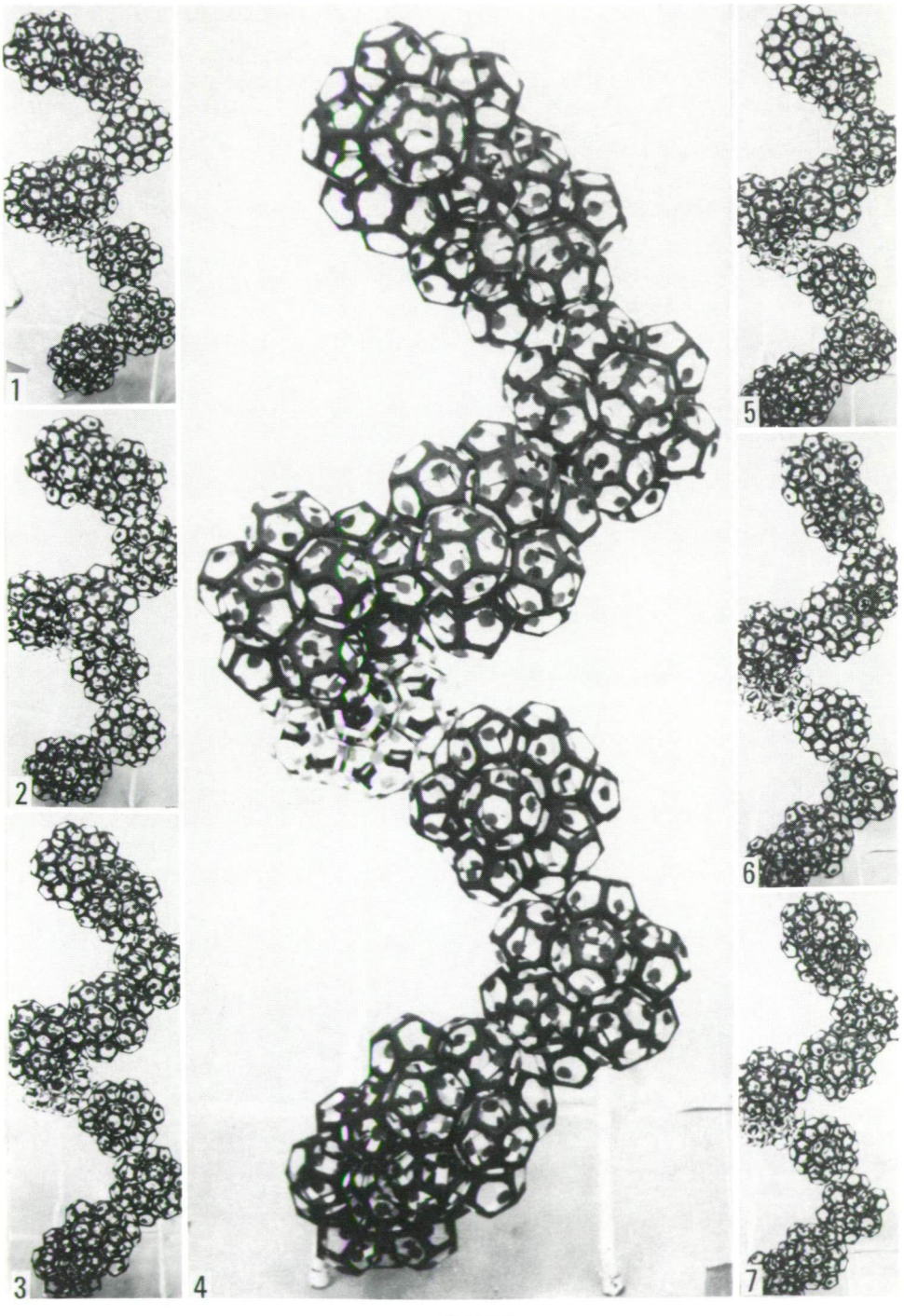

Plate 5.7.

1—7. Heterogeneous secondary helical biopolymer skeleton. The pictures were taken from different positions and magnifications from the same model.

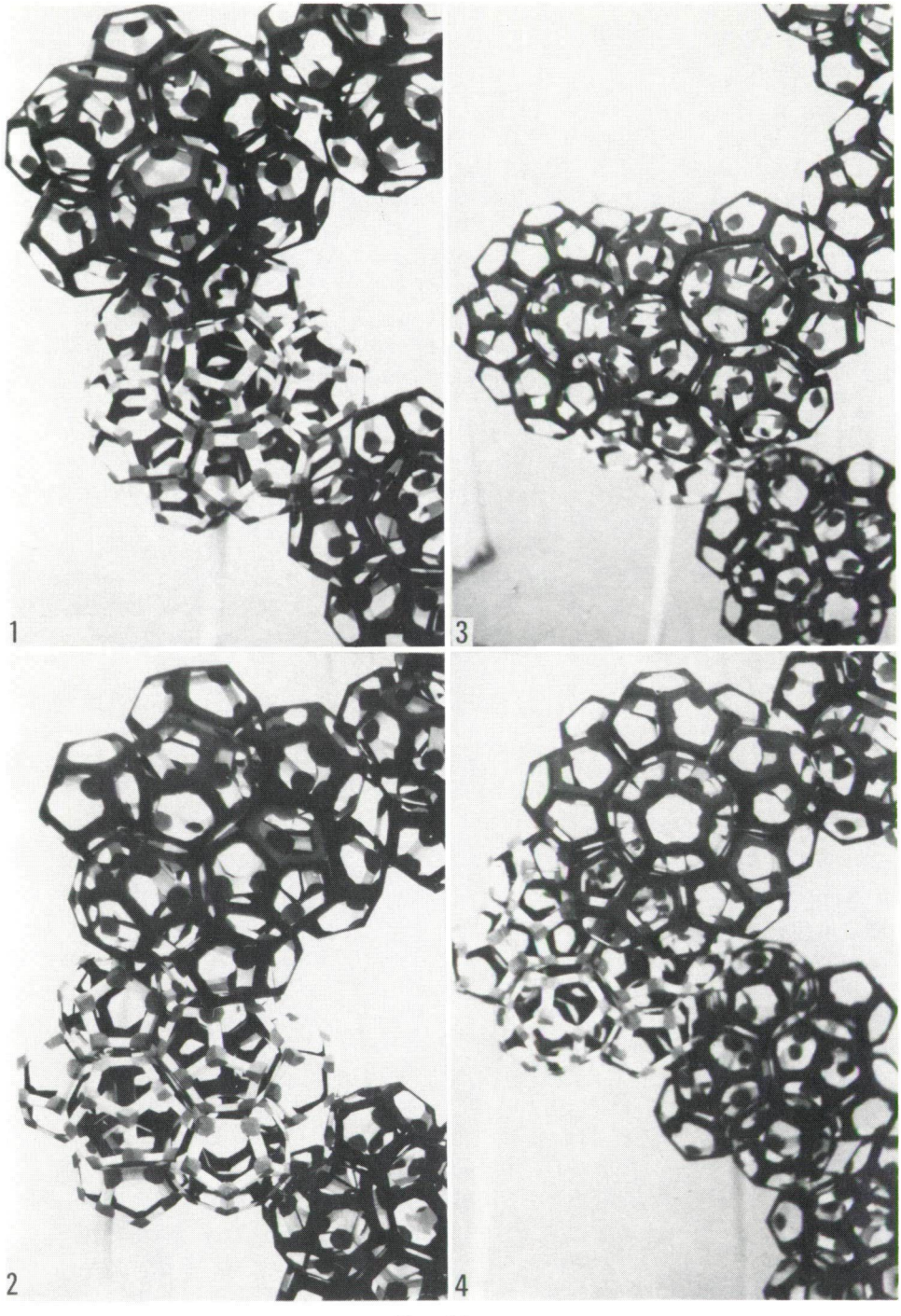

Plate 5.8.

1—4. Details from the connections and points of symmetries of the secondary helical biopolymer model.


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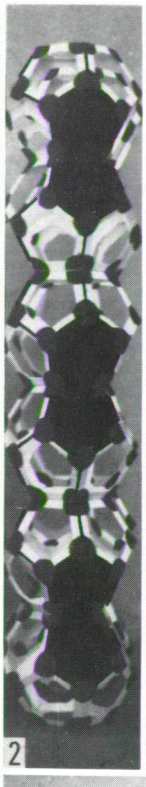
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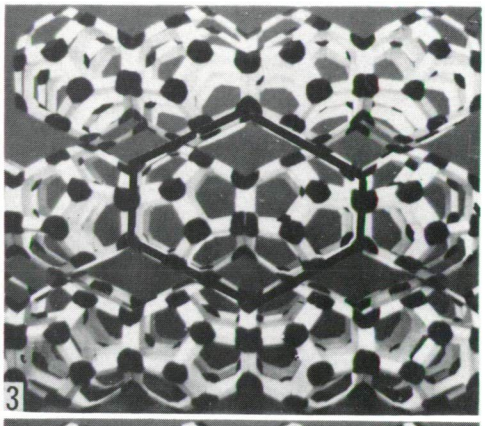
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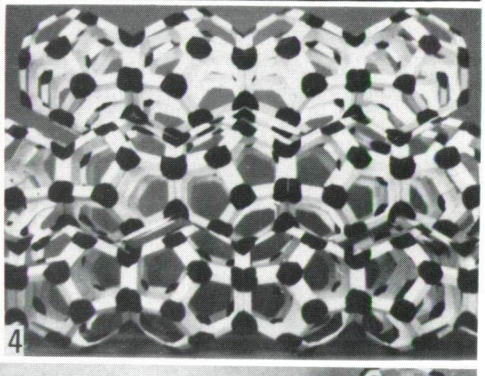
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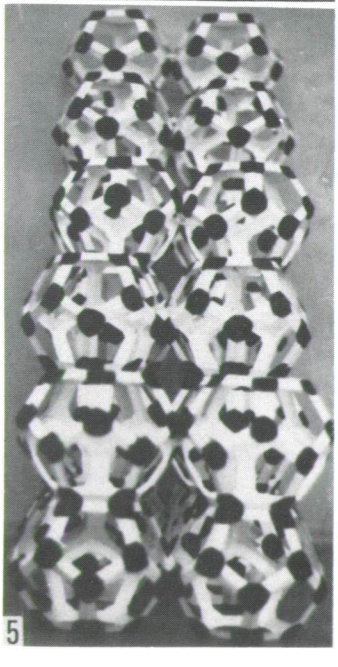
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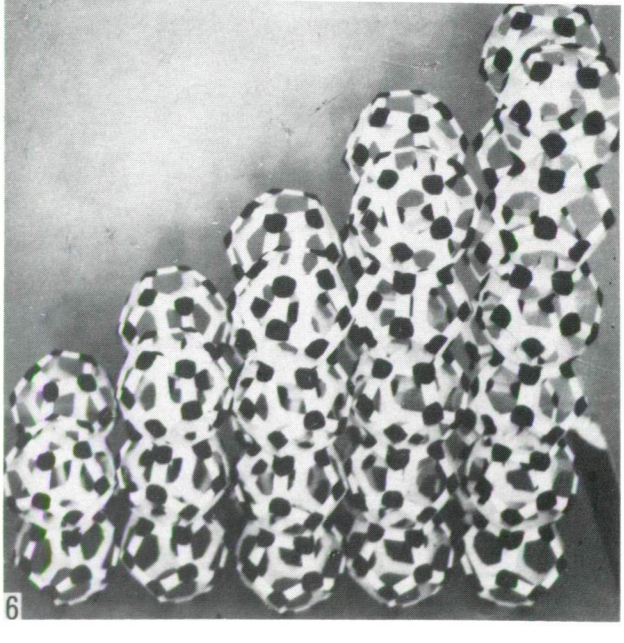
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To complete the large PENROSE—II skeleton we have not enough model but we started this. One PENROSE—I unit was connected to this system, in a completely different manner. Fig. 8 in Plate 5.5. and figs. 1—6 of Plate 5.6. illustrate well this skeleton. The hand of Mrs. BIRÓ—HALÁSZ indicate this ninth PENROSE—I unit, which may be said as the first of the second type of connections in the complete PENROSE—II biopolymer skeleton system. On the basis of our up-to-date knowledge the most important characteristic features of this kind of connection can be summarized in the following:

i. The most important establishment is that this (later those) PENROSE—I unit do not reach or connect to the "Central PENROSE—I" biopolymer unit.

ii. It is an opportunity for two kinds of orientation, corresponding to the two basic positions of the PENROSE—I model, as it is illustrated in Plate 5.4., fig. 1,2. In the case of the connection of fig. 1, the surrounding PENROSE-I type skeleton will be heterogeneous taking its surface. In the case of the so-called periodic position the "surface character" will be homogeneous.

iii. The connections of these units with the previous first surrounding skeletons are not uniform. This problem needs further investigations.

iv. Finally such a PENROSE—II model can be assumed too, when the central unit is not completely connected to the surrounding "shell" composed of PENROSE—I units. All these problems together with the problem of the stabilizing biopolymer system will be investigated later.

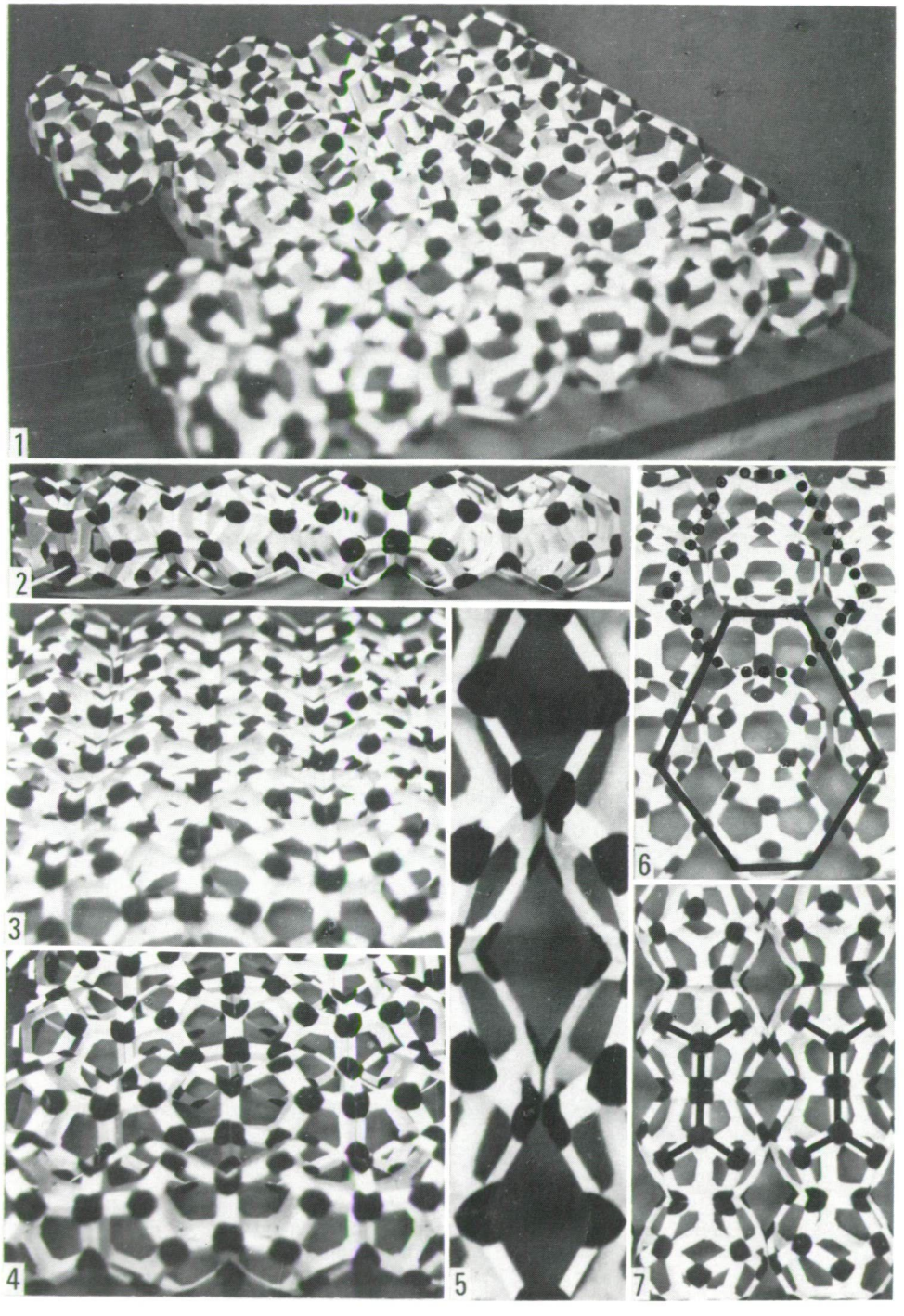
Secondary helical biopolymer skeleton
(Plate 5.7., figs. 1—7, plate 5.8., figs. 1—4)

As it was emphasized in the previous paper the modelling of the helical biopolymer organization will be continuted. In this paper as a new step of the proceeding in this problem the helical model built from PENROSE—I units is presented. To the connections the previously discussed can be said classical, two times three edges method was used. It is necessary to emphasizes that during the building of this model it was observed that there are several opportunities for the connections, which can be resulted in helical systems of different deviation diameter. As the final results of several essays, the illustrated model was completed.

◀ Plate 5.9.

1—6. Quasi-crystalloid modelling of the primary lamellar biopolymer structures.

1. Front view of the model of the staggered lamella. It is necessary to point out that in this view all kinds of symmetries can be observed. The most important biopolymer symmetry configurations are numbered.
2. Lateral view of one primary lamella. The periodicity in the modelling symmetry is identical with those at the primary filament, cf. Plate 5.1., fig. 3.
3. Above view of the two layered lamella model. Worth of mentioning is a peculiar hexagon.
4. Slightly obliquely lateral view of the quasi-crystalloid skeleton of the two -layered lamella. The connections between the two lamellar systems are important at this kind of modelling.
5. Front view of the model of the staggered lamella, but from different angles in contrast to picture 1 of this Plate.
6. General survey picture from this modelling.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The photographs taken from different views well represent the complexity of the biopolymer system in such a biological primary important biopolymer structure. This helical system results important and interesting periodicity in its basically quasi-periodic units. This periodicity is inside the helical system, without alterations inside the helical line. For a basic PENROSE—I unit the sixth one is in a superponal position. The deviation of this helical model is about 168 Å. Pictures of larger magnification (Plate 5.8., figs. 1—4) well illustrates the points of symmetries of this biopolymer model.

Primary lamellar biopolymer structures
(Plate 5.9., figs. 1—6)

It is self-understanding that all biological surfaces are extremely important in several points of view. The delimitation inside or outside the biological systems, and its most important characteristic features were the subject of several studies. It is necessary to cite again as fundamentally important establishments the following:

1. The surface of the exine is anionic (ROWLEY, 1971).

2. "the wall itself is a molecular sieve", p. 449, ROWLEY (1973). After the first discovery of the regular pentagonal polygon biopolymer structure in the pollen exine (KEDVES, 1988), at the first two dimensional scheme for the organization levels of the sporopollenin (KEDVES, 1989, p. 63), the lamellar biological molecular system was also pointed out as an important one.

The three dimensional model was prepared as follows. Two fibrillar units were connected. These so called elementary lamellar model units were prepared by different sizes; the length of 2, 3, 4, 5 and 6 pentagon dodecahedrane units. In this way a staggered lamellar model was completed (Plate 5.9., fig. 6), and investigated by different views. The lateral view of one lamella (Plate 5.9., fig. 2) resulted naturally, the identical periodicity which was established at the primary filament (cf. Plate 5.1., fig. 3). The staggered lamella in front of view of its plane (Plate 5.9., fig. 1 and 6) was investigated by different angles and resulted in interesting and important points of symmetries. In picture no 1 of Plate 5.9. all important systems of symmetry were observed such as rhombus, triangle, hexagon and pentagon. In this way this is another opportunity to explain the results of the non-fivefold rotations, in general the modified MARKHAM rotation method.

From above is photographed a two layered quasi-crystalloid skeleton lamellar model, among the numerous points of symmetry systems and interesting hexagon was pointed out (Plate 5.9., fig. 3). This is in the centrum of the model. The slightly

◀ Plate 5.10.

1—7. Primary modelling of the surface or delimitate quasi-crystalloid layer.
1. General survey aspect of this kind of modelling.
2. Lateral view of this layer.
3, 4. Slightly obliquely lateral view of the surface. These pictures were taken from different angles.
5—7. Points of symmetries of the surface from right angle of the surface plane. The most important configurations are marked.

obliquely lateral view of the two-layered lamella (Plate 5.9., fig. 4) well represents the connections between the two quasi-crystalloid lamellae, different from the above discussed models, cf. Plate 5.9., fig. 1,5 and 6.

The surface of delimitate quasi-crystalloid layer
(Plate 5.10., figs. 1—7)

A uni-layered quasi-crystalloid skeleton was investigated. As a general aspect, fig. 1, in Plate 5.10. is presented. The lateral view is identical with the above discussed ones at the filament (Plate 5.1., fig. 3) and at the uni-layered lamella (Plate 5.9., fig. 2). The pictures taken from different angles of the surface resulted in different systems of points of symmetries (Plate 5.10., fig. 3,4). The perpendicular views are the most interesting and well represent the peculiar characteristic features of this kind of quasi-periodic system. Fig. 5, of Plate 5.10. represents the connections between the two filamental units. The centrum of the middle tetragon is essentially in the right angle of the surface. The previously mentioned hexagonal system (Plate 5.10., fig 6) and the paralell short axes (Plate 5.10., fig. 7) were pointed out.

Discussion and conclusions

I hope that with this paper a remarkable proceeding was made to the knowledge of the quasi-crystalloid biopolymer structures. But further investigations in this respect seem to be necessary, as it was emphasized in connection with the PENROSE-II quasi-crystalloid skeleton. But at this moment we need more information about the stabilizing biopolymer system and start its modellization together with the quasi-crystalloid skeleton. This project will be realized in all probability in the not so far future.

Acknowledgements

The writer is deeply indebted to Dr. I. BAGI, Mrs. I. BIRÓ-HALÁSZ and to Miss E. MARKÓ for their valuable assistance in the preparation and the documentation of these models. This work was financially supported by grant OTKA 1/3, 104.

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6. TEM STUDY OF ULTRATHIN SECTIONS OF THE PARTIALLY DEGRADED WALL OF THE SCLEREIDS OF ARMENIACA VULGARIS LAM.

Short communication

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It was previously emphasized (KEDVES 1991a, b) that our research program about the biopolymer organization of the plant cell wall is not restricted only to the resistant wall of the spores and pollen grains. All kinds of the plant cell wall are to be included in this research program. The extremely thickened cell wall of the sclereids of *Armeniaca vulgaris* LAM. are also considered. As regards the methods of investigations it is necessary to emphasize the following.

1. The dried endocarp of *Armeniaca vulgaris* LAM. was broken in a porcelain mortar. The granules of the endocarp were partially degraded with two kinds of solvents: merkapto-ethanol and diethylamin. Temperature: 30 °C, length of time vary from 1 day up to 5 days.

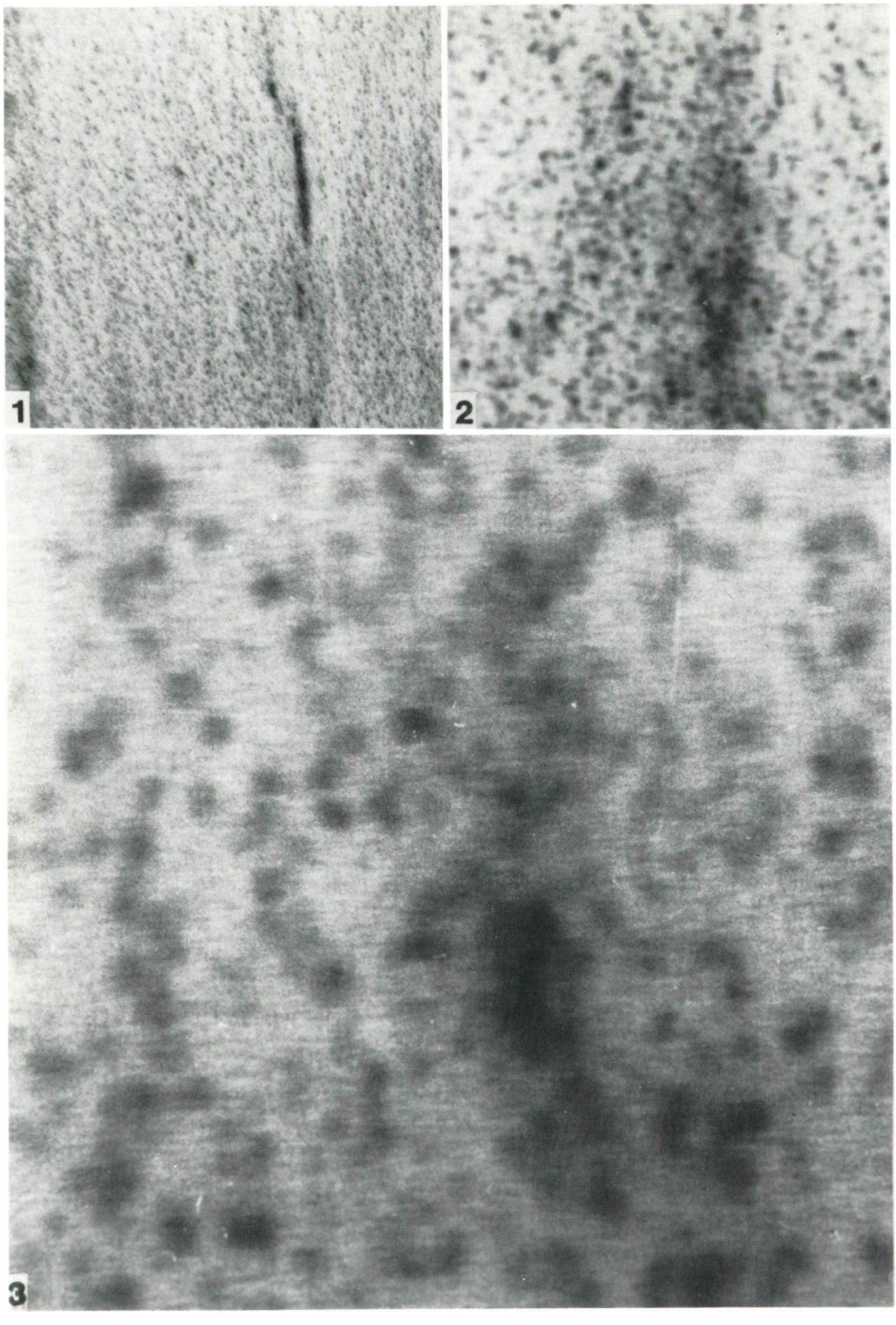
2. For all kinds of the partially degraded cell wall two further methods were used.

2.1. The solvated sclereids were fragmented in a magnetic stirrer for half an hour. The residue was mounted on collodium protected grids, and investigated with transmission electron microscope. A preliminary report on the first results was published; KEDVES and ROJIK (1991). Regular pentagons as basic units, Penrose-like highly organized biopolymer structures were observed. Moreover interesting information was obtained about the stabilizing system of the quasi-crystalloid skeleton, with a peculiar molecular torsion.

2.2. To get complete information from all kinds of partially degraded sclereids, ultrathin sections were also made and investigated with the TEM instrument of the Biological Research Center of the Hungarian Academy of Sciences (OPTON 902),

Plate 6.1. ▶

1–3. *Armeniaca vulgaris* LAM., sclereids.
Experiment No: 954, TEM pictures from the ultrathin sections of the partially degraded secondary wall.
1. Negative no: 1347, 100.000x.
2. Negative no: 1348, 250.000x.
3. Negative no: 1349, Magnification 1 million.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resolution 2.5—3.5 Å. Among the first results the following are presented herein. Taking into consideration the novelty of this kind of researches this paper is "pro parte" a little methodological. For example the results of two experiments are described and discussed in this paper. Experiment No: 954 (Plate 6.1., 1—3) (10 mg fragmented sclereids of endocarp+1 ml merkapto-ethanol, temperature 30 °C, length of time 2 days, washing with distilled water) Experiment No: 962 (Plate 6.2., 1—4) (10 mg fragmented sclereids of endocarp+1 ml merkapto-ethanol, temperature 30 °C, length of time 4 days, washing with distilled water). The residues were fixed with OsO_4 aq. dil., embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made on a Porter Blum ultramicrotome with glass knives.

On Plate 6.1., fig. 1., the finely lamellar system of the cell wall of the sclereids is well illustrated. The biopolymer structure is oriented, forming a fibrillar and/or lamellar system. In the higher magnified pictures (Plate 6.1., fig. 2,3) this orientation is not so characteristic. From another point of view, concerning to fig. 3, Plate 6.1., at magnification 1 million we can emphasize the following.

There are globular units of 4—8 Å in diameter. Their arrangement is more or less linear or irregular. This globular system is embedded in a fine network-like matrix. The meshes of this biopolymer structure is 2.5—4 Å. In fig. 3. it is well shown that the partial degradation is not in an advanced phase. In this respect the solvatation during 4 days (experiment No: 962) with the same solvent is extremely clear. The molecular system of the "matrix" is very characteristic. The larger globular units of 4—8 Å in diameter are also much more characteristic than at the previous experiment. Moreover at this experiment it is well shown that these larger units are composed of smaller ones. Taking into consideration "some possible structures of polycondensed aromatic molecules" published by OBERLIN, BOULMIER, and VILLEY (In: DURAND, ed., 1980), p. 216, particularly the formula "m", Fig. 7.20., it is probable that these biopolymer structures are the components of the aromatic stabilizing system. Following the above mentioned formula the size of three alternate benzine ring is 7.1 Å, one benzine ring is encircled with six further ones. As regards the low magnified pictures (Plate 6.2., figs. 1—3) the fine lamellar ultrastructure of the cell wall is well shown at the lowest magnification, at 25.000x. The complete "monographic elaboration" of the full experimental data will be the subject of further publication.

Acknowledgements

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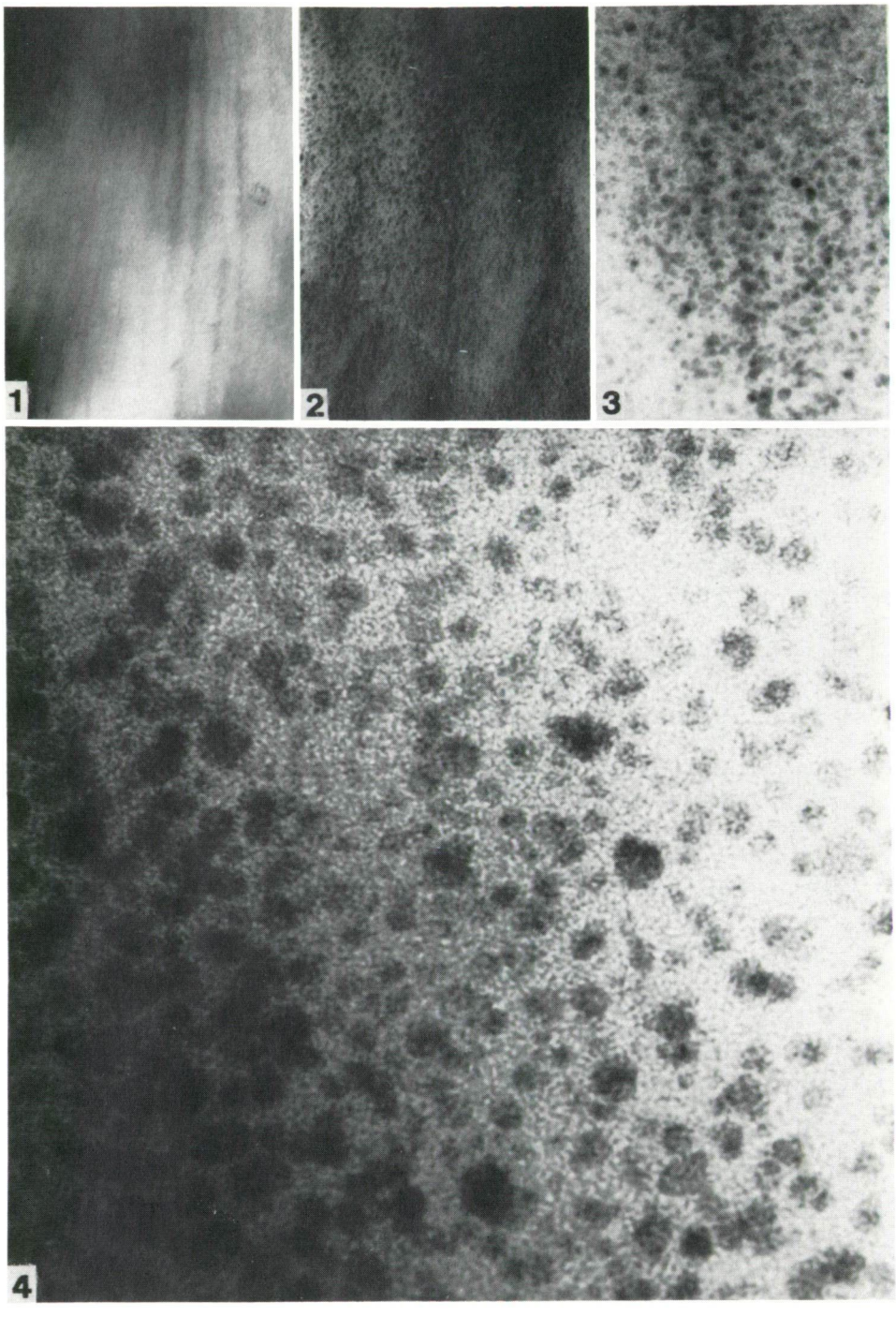
Plate 6.2. ▶

1—4. *Armeniaca vulgaris* LAM., sclereids.
Experiment No: 962, TEM pictures from the ultrathin sections of the partially degraded secondary wall.
1. Negative no: 1373, 25.000x.
2. Negative no: 1374, 100.000x.
3. Negative no: 1375, 250.000x.
4. Negative no: 1376, Magnification 1 million.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Chronicle

Visiting scientists

Dr. M. T. FERNANDEZ MARRÓN (U.E.I. of Paleontology, Institut of Economic Geology, C.S.I.C.–U.C.M., Madrid, Spain).

During her stay in Szeged (11. 6. 1991–21. 6. 1991) the 2nd contribution of this number was completed. Outside of our laboratory she visited the EM Laboratory of the Institute of Biophysics (Hungarian Academy of Science) and continued discussions about the problems of the TEM methods with Dr. Á. PÁRDUTZ.

Dr. B. JELEN (Geological Survey of Ljubljana, Ljubljana, Republic of Slovenia).

(15. 12. 1991–18. 12. 1991) A remarkable number of slides containing fossil palynomorphs from Tertiary sediments of Slovenia were investigated by the LM method. Among the spore-pollen assemblages from different localities the so-called "Dorog type" middle Eocene one was also identified. Scientific co-operation was planned and organized. Dr. B. JELEN continued fruitfull discussions with Prof. Dr. T. SZEDERKÉNYI (Department of Mineralogy, Geochemistry and Petrology of the J. A. University).

International laboratory activity

On the XII[e] Symposium APLF; Biogeographie et Palynologie (Caen, 23–27 September 1991) Dr. M. KEDVES presented his lecture as follows: "Les modeles à trois dimension de l'organisation biopolymère du sporoderme". Visiting scientist during two weeks in Spain (8. 10. 1991–22. 10. 1991) fruitful discussions were continued with the professors and researchers of the following institutions: Dr. J. CIVIS LLOVERA, Dr. F. VALLE HERNÁNDEZ, Dr. M. R. RIVAS CARBALLO, Dr. J. ABEL FLORES (Departamento de Geologia, Facultad de Ciencias, Universidad de Salamanca), Dr. J. DE PORTA, Dr. N. SOLÉ DE PORTA, Dr. JORDI MARTINELL, Dr. C. MARTIN CLOSAS, Dr. R. DOMENECH (Universitat de Barcelona, Departament de Geologia Dinamica, Geofisica i Paleontologia, Facultad de Geologia, Zona Universitaria de Pedralbes), Dr. J. A. SEAONE-CAMBA, Dr. M. SUAREZ-CERVERA, J.

MÁRQUEZ PEREIRA (Universidad de Barcelona, Facultad de Farmacia, Departamento de Botanica). Dr. C. ALVAREZ RAMIS, Dr. M. T. FERNANDEZ MARRÓN (Departamento de Paleontologia, Facultad de Ciencias Geológicas, Ciudad Universitaria, Madrid). Lectures: in Salamanca on the 11. 10. 1992 at 11^{h}, in the Aula D—2, Biochemistry; in Barcelona on the 15. 10. 1992 at $17^{30'}$, in the Aula 32, Geological Faculty. The subject of both lectures was the same: the biopolymer organization and the modellisation of the sporoderm wall. The newly organized common research program was mentioned in the 2nd contribution of this number.

Photograph

Dr. M. T. FERNANDEZ MARRÓN in the Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J. A. University, Szeged. The picture was taken by Dr. É. SIPOS—KEDVES.

Laboratory meetings

1. 3. 1991, speaker: VÉR, A.: Modelling of the quasi-crystalloid systems and its importance in the biological structures.
22. 3. 1991, speaker: KEDVES, M.: *Sequoia* Woodland (California) and *Taxodium* Swamp (Florida), U.S.A.
12. 4. 1991, speaker: KEDVES, M.: Report from the participations on international scientific meetings (Lucknow, Uttar Pradesh, India) and Tenerife, Islas Canarias, Spain.
17. 5. 1991, speaker: VÉR, A.: Biopolymer organization of the biological constituent of the alginite *(Botryococcus braunii* KÜTZ.*)*.
22. 11. 1991, speaker: KEDVES, M.: Report from the *"Hamamelidae* Symposium" (Reading, U.K.), and from Barcelona and Tarragona, Spain.
29. 11. 1991, speaker: KEDVES, M.: Report from the scientifical contacts, and from the University and Botanical Garden from Lisbon.

Teaching program of the Laboratory

During the last two semesters the following lectures were delivered:
Introduction to the plant micropaleontology of pre-Quaternary deposits, 1+2 hours weekly.
Organizations levels of the biopolymer system of the plant cell wall, 1+2 hours weekly.
Introduction to the Supernova Theory, 1 lecture weekly. Theory of the evolution and its natural phylosophical relations, 1 lecture weekly.

Laboratory party

At the occasion of the appearance of the first number of the Plant Cell Biology and Development on 26. 4. 1991 at 2 hours p.m. an exclusive party was held in the laboratory. Persons who helped the laboratory organization and the talent scouting fonction of the laboratory were invited: Dr. J. BÁNFALVY, M. BOGÁTHY-EKE, Dr. B. CSÁKÁNY, Dr. I. GYÉMÁNT, M. JURAY, Dr. E. SZALAMIN. From the Department of Botany Dr. I. BAGI and L. TÓTH-SOMA. The young people were represented by the foundenter students of the laboratory: E. FARKAS and A. TÓTH.

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